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The Journal of the Indian Botanical Society

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FURTHER INFORMATION ON *HOMOXYLON RAJMAHALENSE* SAHNI*

(With 4 plates and 18 text-figures)

BY J. HSÜ AND M. N. BOSE

Birbal Sahni Institute of Palæobotany, Lucknow

(Received for publication on June 15, 1951)

INTRODUCTION

Homoxylon rajmahalense is one of the most interesting petrified fossils described by Professor Sahni (1932) from the Jurassic of the Rajmahal Hills of India. There was a little doubt about the age of the original specimen as it was recovered by V. Ball of the Geological Survey of India from an unknown locality of the Rajmahal Hills (Behar) many years ago.

The original specimen was only a piece of secondary wood. Its interest lies entirely on growth rings and on the pitting of the radial walls of tracheids, including both scalariform and multiseriate. Professor Sahni once thought that it was a fossil angiospermous wood, but he hesitated to decide its affinity as it might actually belong to a gymnospermous plant (Sahni, 1932, p. 14).

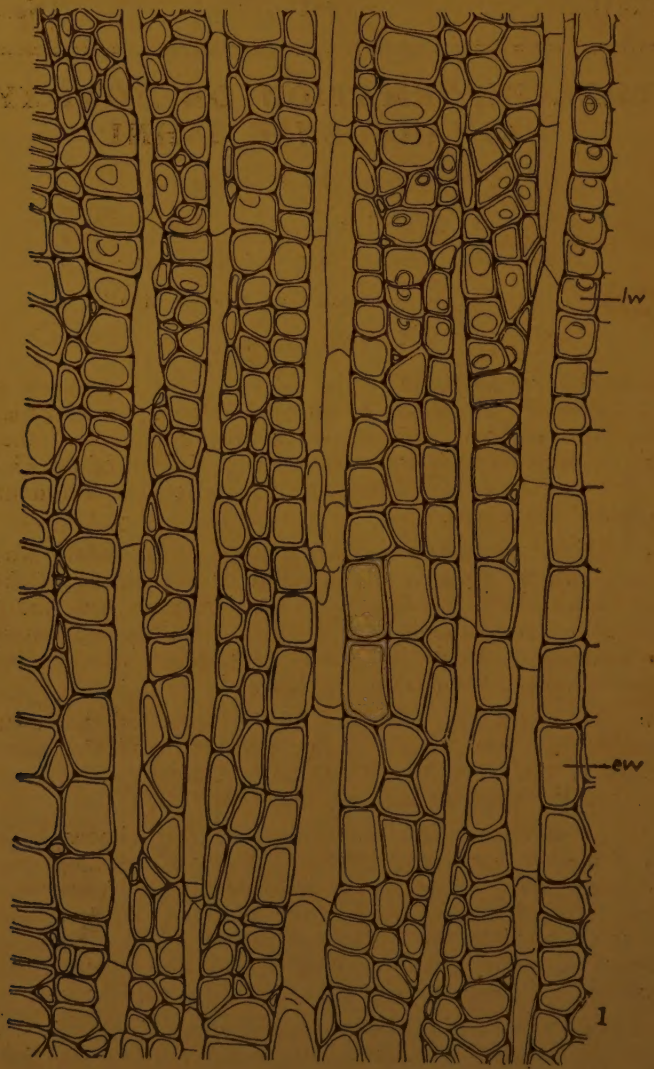
Later on, Dr. K. M. Gupta (1933–34) suggested that comparison was possible with the woods of Bennettitales, and Professor Sahni (1938) himself had also agreed to that. Unfortunately no evidence has so far been found to support this view.

The present paper deals with some other specimens collected in 1948 by Professor Sahni and others in a party including one of the authors from Amarjola in a classical locality of Amrapara in the Rajmahal Hills of Behar, India, from where a *Williamsonia* female flower was collected by G. V. Hobson (Sahni, 1932). These are three small pieces of poorly petrified stems. They are rather crumbly, but after boiling in Canada balsam they are quite easy to cut.

On careful examination the secondary wood of these fossils closely resembles the original specimen described by Professor Sahni, but unlike the latter they are complete, having a large central pith, a broad

* This paper was read before the Palæobotanical Section of the Seventh International Botanical Congress, Stockholm, on July 10, 1950.

zone of xylem, phlœm and bark. In structure they can be closely compared with *Bucklandia*, a *Williamsonia* stem covered with leaf bases, found in the same locality. So the present investigation not only settles the geological age of the original specimen, but also shows that *Homoxylon rajmahalense* is not an angiospermous wood but a Bennettitalean wood.



TEXT-FIG. 1. Part of secondary wood in transverse section showing well-marked growth rings with late wood (*lw*) greatly developed as compared with early wood (*ew*). Slide J 4, × 240.

Description

All the specimens investigated were rather small and fragmentary. The biggest one shown in Pl. I, Fig. 1, measuring 4·6 cm. in length and 18 mm. in diameter. Their surface appeared uneven, marked with broken scars here and there. This suggests that they were slender stems or branches but had lost their leaf bases through cork formation. On careful examination all the specimens show similar characters of xylem, phlœm, and pith. Thus it is clear that they all belong to the same species, but for the sake of safety the following description is restricted only to the biggest specimen.

The transverse section (Pl. I, Fig. 2) shows a big central pith surrounded by a broad zone of xylem, phlœm and bark. Nearly all the xylem tissues are secondary derived from a poorly preserved cambium in nine successive years. Broad medullary rays, about 5–10 in number seen in a cross section, deeply intrude into the xylem ring (Pl. I, Fig. 3).

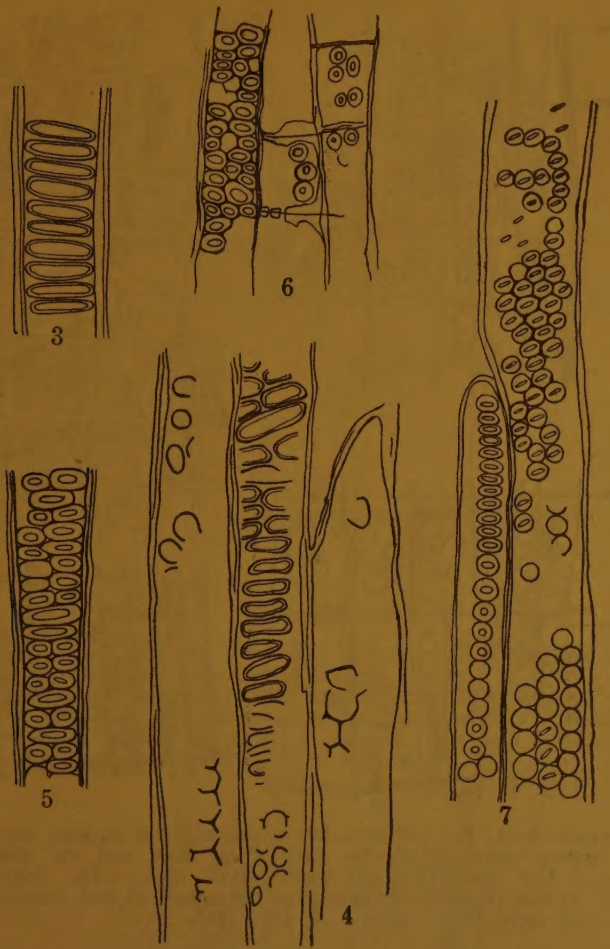
Pl. I, Fig. 4 and Text-Fig. 1 show a part of the secondary xylem in transverse section. It is pycnoxylic. So far no xylem parenchyma has been observed. The tracheids are mostly quadrangular in section and compactly arranged in radial rows. They are separated by the xylem rays with 1–5 cells at one interval. The tracheids of the early wood have a bigger diameter and thinner wall, in contrast with those of the late. But as a whole the late wood is more developed than the early, and occupies about two-thirds of the whole growth ring. The xylem rays are uni- to biseriate, but occasionally triseriate ones have also been seen.

The tangential longitudinal sections (Pl. II, Fig. 10 and Text-Fig. 2) show that the tracheids of secondary wood reach a length of about 2 mm. Pits are not visible on the tangential walls. Uniseriate rays are the commonest, but biseriate ones also occur frequently. A few triseriate rays are also found. They are 1–42 cells in height and the cells are slightly higher than their breadth as seen in tangential section. Usually the marginal cells are bigger than the rest. A number of bordered pits have been observed in the tangential walls of ray cells (Pl. II, Fig. 11 and Text-Fig. 15). Usually they are not well preserved. Frequently only pit cavities are visible (Text-Fig. 14).

The radial section of the secondary wood exhibits some points of great interest. It appears that the late wood (Text-Fig. 8 and Pl. I, Fig. 6, *lt*) possesses tracheids mainly with uniseriate round bordered pits, but early wood shows various types of pitting, ranging from scalariform to multiseriate on the radial surfaces of tracheids (Pl. I, Fig. 5; Pl. II, Fig. 8; Text-Figs. 3–7). The slender tracheids are often with scalariform pitting (Text-Fig. 3), while the wider ones have multiseriate bordered pits ranging from horizontally elongated to circular. The circular pits are 3–4- (or even 5-) seriate, and tend to be compactly arranged in hexagons. The pores appear to be elongated, obliquely arranged and parallel to one another.

TEXT-FIG. 2. A part of secondary wood in tangential section showing uni- to partly triseriate rays. Slide J 11, × 195.

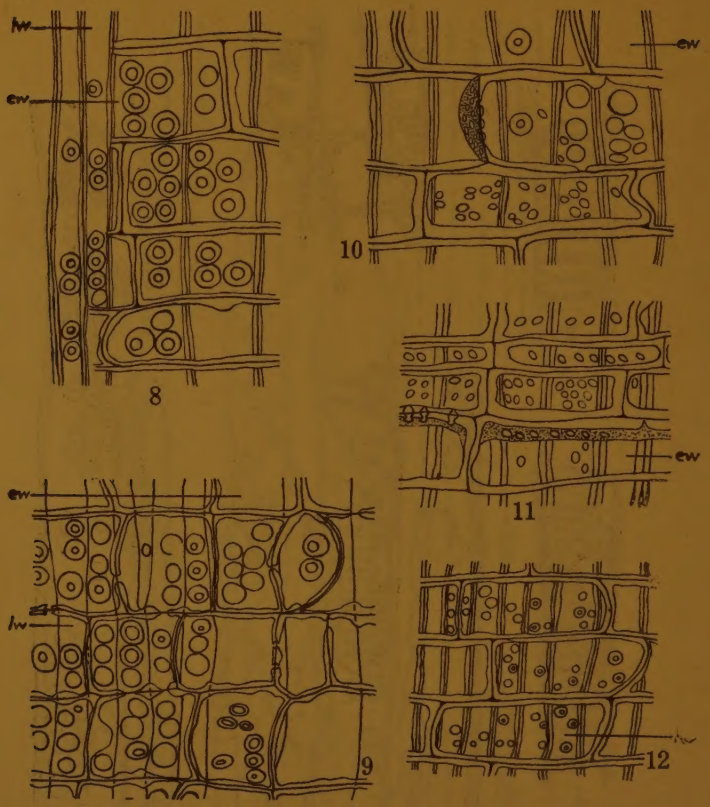
The ray cells are 2–5 times as wide as they are high. The end walls may be vertical or oblique. Pits seen in the radial walls are of both simple and bordered types (Text-Figs. 8–12). Their size varies a great deal, but the bordered ones are usually bigger. Usually the border is not well preserved. Some non-bordered pits shown in Text-Figs. 8–10 evidently were not originally simple in nature. The



TEXT-FIGS. 3–7. Parts of early wood in radial sections, showing various types of pitting occurring on the walls of tracheids. Fig. 3. Tracheid with scalariform bordered pits; Figs. 4 & 5. Tracheids with transitional bordered pittings; Figs. 6 & 7. Late tracheids showing multiseriate bordered pits. Fig. 3, Slide J 8, × 523; Fig. 4, Slide J 17, × 400; Figs. 5–7, Slide J 17, × 265.

number of bordered pits in a cross field varies from one to seven or more. Real simple pits are quite small, numbering 1–12, or even more, in a field (Text-Figs. 10 and 11). Occasionally both simple and bordered pits have been found in the same field. Pittings on transverse and tangential walls of ray cells are also shown in Text-Figs. 10 and 11. Apparently all the walls of ray cells are with pits, simple or bordered or both (Pl. II, Fig. 9 and Text-Figs. 9–15).

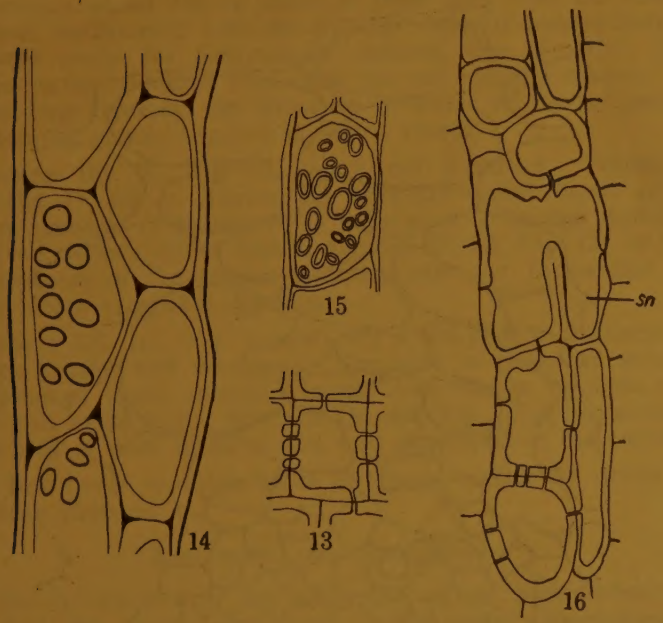
The pith of the figured specimen measures about 8 mm. in diameter. It consists mainly of parenchymatous cells but with hundreds of



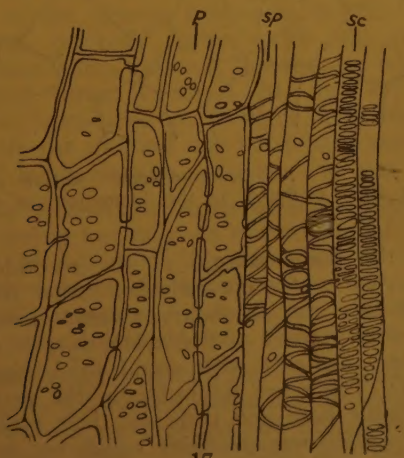
TEXT-FIGS. 8–12. Parts of secondary wood in radial sections showing various types of pitting occurring on the walls of ray cells, and the pittings of the tracheids of late wood (*lw*) and early wood (*ew*). The dotted parts in Text-Figs. 10 and 11 show the pittings of the tangential and transverse walls of the ray cells respectively. Slide J 17, all × 265.

sclerotic nests scattered in it. In cross sections the parenchymatous cells are isodiametric (Pl. III, Fig. 14) with well-developed intercellular spaces. Longitudinal sections show that these cells are quadrangular to rectangular in shape (Pl. III, Fig. 15 and Text-Fig. 17, *p*) with many small simple pits scattered over all the walls. Cells of the sclerotic nests are thick-walled with dark contents. In cross section these are 1–20-celled, quite compact (Pl. III, Fig. 14), and with no intercellular spaces. Longitudinal sections show that they are elongated cells, of various calibres, connected in vertical series (Pl. III, Fig. 15 and Text-Fig. 16, *sn*). Sometimes they are forked in longitudinal section. Only simple pits are found on their walls.

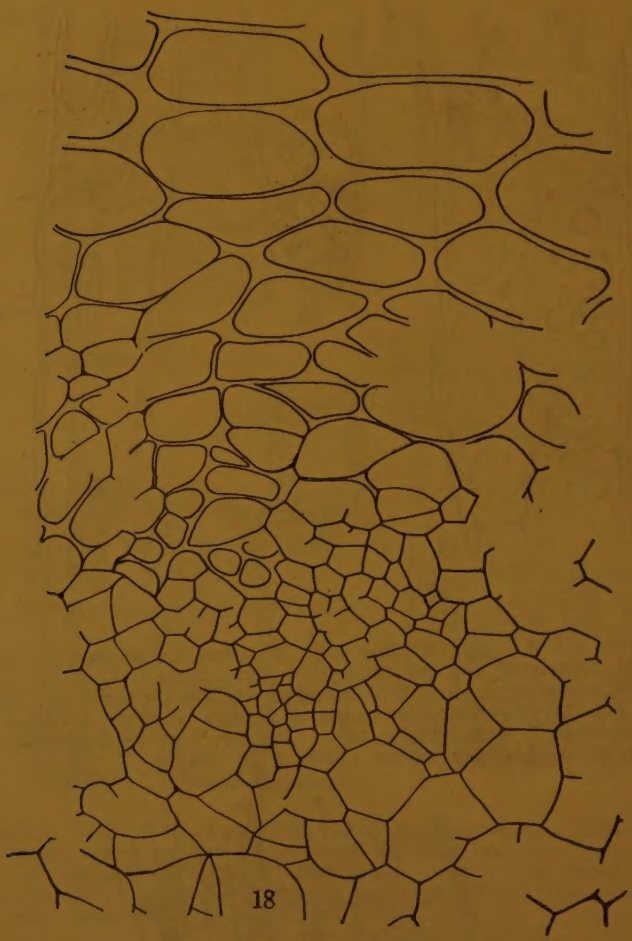
The primary xylem is endarch, but not so easily recognisable in transverse sections. Occasionally some groups of small polygonal cells, about 20–30 in number, presumably the primary xylem, are seen



TEXT-FIGS. 13–16. Fig. 13. Ray cells, in radial section showing simple pits on both the transverse and the tangential walls. Slide J 19, × 265. Fig. 14, Ray cells, in tangential section. The bordered pits are not preserved. Slide J 11, × 725. Fig. 15. Ray cells, in tangential section, showing bordered pits on the tangential wall. Slide J 11, × 545. Fig. 16. Part of a sclerotic nest (*sn*) in the pith in longitudinal section. Slide J 13, × 159.



TEXT-FIG. 17. Inner part of wood, with adjacent cells of the pith (*p*), in radial section. *sp*, spiral tracheids of protoxylem and *sc*, scalariform tracheids. Slide J 9, × 265.



TEXT-FIG. 18. Part of pith in transverse section showing a group of small cells occurring there probably in the formation of an internal periderm or tuber. Slide J 11, × 370.

situated next to the inner margin of the secondary xylem wedges (Pl. II, Fig. 12). Walls of these cells are rather thin compared with those of the pith and the secondary xylem. Longitudinal sections show that the innermost part of the xylem ring is composed of 4 or 5 layers of slender tracheids with spiral thickenings on their walls (Text-Fig. 17, *sp*). These are undoubtedly the protoxylem elements. Simple pits are visible here and there on their walls. Next elements outwards are the scalariform tracheids which are broader, and might be a part of the secondary xylem.

In the pith, quite apart from the xylem wedges (Pl. II, Fig. 13; and Text-Fig. 18), there are a few groups of very small cells. Each

group contains about 100 or more of these cells. All the cells are thin walled, isodiametric with the smallest elements located centrally. Trials were made for longitudinal sections, but in vain. Serial transverse sections indicate that these are not vascular tissues. It is likely that they represent the formation of a sort of internal periderm or a tuber, as usually happens in recent cycads.

Cambium (Pl. III, Fig. 17, *cm*) is not preserved. Phlœm ring (Pl. III, Fig. 17, *ph*) is marked by alternate radial bands of rays and other elements of phlœm. Ray cells seem to be thin walled and ill preserved. Rows, about nine in number (Pl. III, Fig. 17), of heavy walled elements of the phlœm alternate with the thin-walled ones regularly in the tangential direction.

Pericycle (Pl. III, Fig. 16,) is only a few cells in thickness, but the cortex is quite well developed. The latter is composed mainly of parenchymatous tissue, though sclerotic nests similar to those of the pith are also found in it. Periderm (Pl. III, Fig. 16, *pr*) consisting of separate arcs of cork tissue is also well developed in the cortex.

No leaf traces or other kinds of tissues outside the cortex have so far been observed.

Discussion and Conclusion

In the introduction of this paper the authors have already mentioned that the original specimen of *Homoxylon rajmahalense* was a piece of secondary wood, lacking both the inner and outer tissues. Its interest lies entirely on growth rings with late wood more developed than the early and on its peculiar tracheids, which exhibit various types of pitting on their radial surfaces, including both scalariform and multiseriate.

The structure of the present specimens, so far as the secondary xylem is concerned, perfectly agrees with the original specimen described by Professor Sahni (though the former exhibit more details than the latter) which leaves no doubt that they are identical. Most probably the present specimens are younger or belong to a twig only nine years old, while the original one must have come from an older and lower stem of many decades.

It appears that *Homoxylon rajmahalense* is characterised by its great variation of pitting not only on the raidal walls of tracheids but also on the walls of ray cells. The structure of the growth rings, however, is not so unusual. Evidently many Jurassic woods found in the Rajmahal Hills examined by the writers show the same feature, having late wood more developed than the early.

As to the nature and classification of *Homoxylon rajmahalense* judged only by the above description the present specimens still do not exclude Magnoliaceæ, nor do they exclude Bennettitales, as previously discussed by Professor Sahni (1932, 1938), and Gupta (1933, 1934). But so far the records of fossils are concerned this locality is abundant in petrified *Williamsonia* flowers, both male and female, leaves of *Ptilophyllum*, *Dictyozamites* and *Anomozamites* types, *Nipaniophyllum*

and *Pentoxylon* and many other gymnospermous cones, branches, and leaves. No associated leaf resembling the dicotyledonous ones has ever been discovered. This fact suggests that *Homoxylon rajmahalense* is possibly not an angiospermous wood. The authors have already studied some *Williamsonia* stems of *Bucklandia* type, covered with leaf bases. These stems are correlated with *Ptilophyllum* leaves. Structure of these stems is just like that of the present specimens (Pl. IV, Figs. 18–20) except that the secondary wood is mainly composed of scalariform tracheids and the perimary xylem and the internal periderm are better developed. A part of a transverse section of *Williamsonia* stem is shown in Pl. IV, Fig. 18 which is closely comparable with that of *Homoxylon rajmahalense* figured in Pl. I, Fig. 2. The tangential and transverse sections of the secondary wood of *Williamsonia* stem (Pl. IV, Figs. 19–20) are just like those of *Homoxylon rajmahalense* figured in Pl. II, Fig. 10 and Pl. I, Fig. 4 respectively. In radial section, the secondary wood shows that the tracheids are mainly scalariform, but multiseriate ones are also found (Pl. IV, Fig. 21). The ray cells show some big pits which might be different from those of *Homoxylon rajmahalense*. This difference, if at all, should be considered as a specific character of a certain *Williamsonia* species other than that represented by the secondary wood of *Homoxylon rajmahalense* type. Its pith also possesses numerous sclerotic nests (Pl. IV, Fig. 22) closely comparable with those of *Homoxylon rajmahalense* (Pl. II, Fig. 14). All these characters are still under investigation and will be described in detail in a separate paper. At present the authors do not intend to presume that *Homoxylon rajmahalense* is definitely identical with *Bucklandia*. But the comparison is so close as to leave hardly any doubt about *Homoxylon rajmahalense* being the secondary wood of a Bennettitalean plant. However, possibilities are not excluded that *Homoxylon rajmahalense* might be a part of *Bucklandia* which had lost its leaf bases through cork formation.

Summary

This paper deals with some specimens identical with *Homoxylon rajmahalense* Sahni collected from Amarjola of the Rajmahal Hills of India.

The original specimen of *Homoxylon rajmahalense* was merely a piece of secondary wood. Its interest lies entirely on growth rings, and its peculiar tracheids. These tracheids show various types of pitting including both scalariform and multiseriate on the radial walls. It closely resembles the secondary wood of certain dicots without vessels, such as *Trochodendron*, *Tetracentron* and *Drimys* which are placed in or near the Magnoliaceæ.

The present specimens are complete, having a large central pith, a broad zone of xylem, phlœm and bark. The original one must have come from the lower part of an old stem.

Among the tissues of pith are numerous sclerotic nests. Primary xylem is endarch. All the details of the secondary wood of the new fossils agree perfectly with the original specimen. The cambium is not

preserved, but the phlœm is present with heavy walled elements alternating with the thinner-walled ones with marked regularity in tangential direction. The phlœm rays are usually ill-preserved leaving big cavities alternating with the bands of phlœm. Next to phlœm are pericycle and cortex. The periderm occurring in the cortex consists of separate arcs of cork tissues.

No leaf traces or other kinds of tissues outside the periderm have so far been observed. A few irregularly distributed masses of small cells are situated in the peripheral region of the pith, which are believed to represent the formation of a sort of internal periderm. Close comparison in anatomy has been made with *Bucklandia* (a *Williamsonia* stem) found in the same bed as the present specimens. This indicates *Homoxylon rajmahalense* as being the secondary wood of a Bennettitalean plant.

We wish to express our thanks to Professor T. M. Harris of Reading for some valuable snggestions during the preparation of this paper.

Literature Cited

Gupta, K. M. 1933. The Homoxyleæ and the ancestry of Angiosperms. Curr. Sci. 2: 129–30.

———. 1934. On the wood anatomy and theoretical significance of Homoxylous angiosperms. Journ. Ind. Bot. Soc. 13: 71–101.

Sahni, B. 1932. *Homoxylon rajmahalense*, gen et sp. nov., a fossil angiospermous wood devoid of vessels, from the Rajmahal Hills, Behar. Palæontologia Indica, New Ser. 20: No. 2.

———. 1932. A petrified Williamsonia (*W. sewardiana*) sp. nov., from the Rajmahal Hills, India. Palæontologia Indica, New Ser. 20: No. 3.

———. 1938. Recent advances in Indian Palæobotany. Pres. Add., Botany Section, Proc. 25th Ind. Sci. Cong. Jubil. Sess., Calcutta. 2: 133–76; and Lucknow University Studies, Fac. Sci. 2: 1–100.

Explanation of Plates

Plate I

Homoxylon rajmahalense

Fig. 1. The specimen from which all the sections were made, × 1.

Fig. 2. Transverse section, showing the large pith and the broad zone of xylem, a ring of phlœm and partly preserved cortex. Slide J 5, × 3·5.

Fig. 3. Part of transverse section, showing part of pith and some wedges of xylem. Slide J 4, × 70.

Fig. 4. Part of transverse section, showing well marked growth rings with late wood greatly developed as compared with early wood. Slide J 6, × 70.

Fig. 5. Inner part of xylem, with adjacent cells of pith, in radial section. *p*, pith; *sp*, spiral tracheids of protoxylem; *sc*. scalariform tracheids. Slide J 9, × 460.

Fig. 6. Part of secondary xylem in radial section, showing late tracheids (*lt*) with uniseriate bordered pits. Slide J 9, × 280.

PLATE II

Homoxylon rajmahalense

FIG. 7. Part of secondary xylem in radial section, showing scalariform tracheids (*sc*) of the early wood. Slide J 17, × 280.

FIG. 8. Part of secondary xylem in radial section, showing some early tracheids with multiseriate bordered pits. Slide J 9, × 460.

FIG. 9. Part of secondary xylem in radial section, showing the bordered pits (*bp*) on the walls of ray cells. Slide J 23, × 660.

FIG. 10. Part of secondary xylem in tangential section, showing the uni- to biseriate rays. Slide J 11, × 55.

FIG. 11. Part of secondary xylem in tangential section, showing bordered pits crowded on the walls of ray cells. Slide J 11, × 660.

FIG. 12. Part of the stem in transverse section, showing a possible primary xylem strand (*px*) occurring at the edge of the pith (*p*). Slide J 2, × 280.

FIG. 13. Part of pith in transverse section, showing a group of small cells occurring there probably in the formation of an internal tuber. Slide J 1, × 100.

PLATE III

Homoxylon rajmahalense

FIG. 14. Part of pith, in transverse section, showing sclerotic nest (*sn*). Slide J 5, × 280.

FIG. 15. Part of pith, in longitudinal section, showing sclerotic nests (*sn*) and ordinary pith cells (*p*). Slide J 10, × 120.

FIG. 16. Part of a transverse section of the stem, showing the broad zone of phlœm (*ph*), the cortex (*c*) and the perlderm (*pr*). Slide J 1, × 25.

FIG. 17. Part of a transverse section of the stem, showing the badly preserved cambium (*cm*), and the phlœm (*ph*). The thicker-walled elements of phlœm alternate with the thinner-walled with marked regularity in tangential direction. Slide J 8, × 55.

PLATE IV

A *Williamsonia* stem

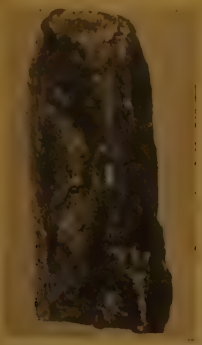
FIG. 18. Part of a transverse section, comparable with that of *Homoxylon rajmahalense* (Pl. I, fig. 2). Slide J 28, × 20.

FIG. 19. Part of secondary wood in tangential section, comparable with that of *Homoxylon rajmahalense* (Pl. II, Fig. 10). Slide J 29, × 90.

FIG. 20. Part of secondary wood, in transverse section, comparable with that of *Homoxylon rajmahalense* (Pl. I, Fig. 4). Slide J 32, × 90.

FIG. 21. Part of secondary wood in radial section, showing scalariform tracheids and ray cells. Slide J 30, × 280.

FIG. 22. Part of pith, in transverse section, comparable with that of *Homoxylon rajmahalense* (Pl. III, Fig. 14). Slide J 31, × 25.



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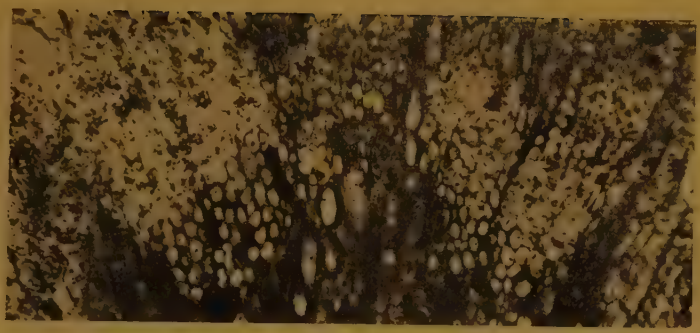

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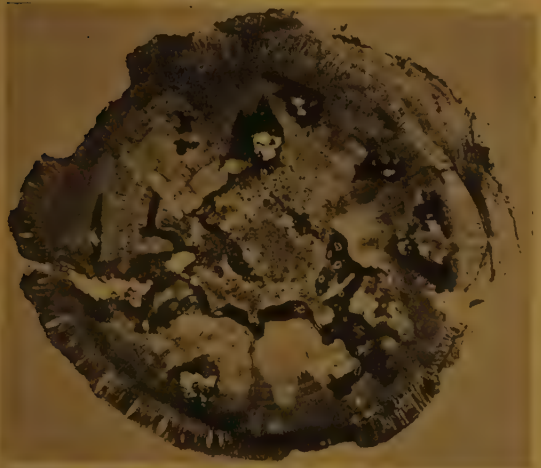

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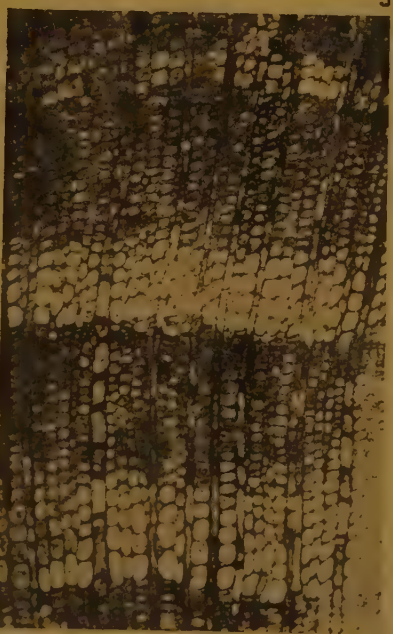

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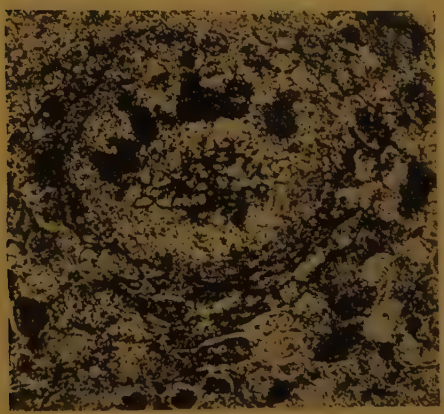
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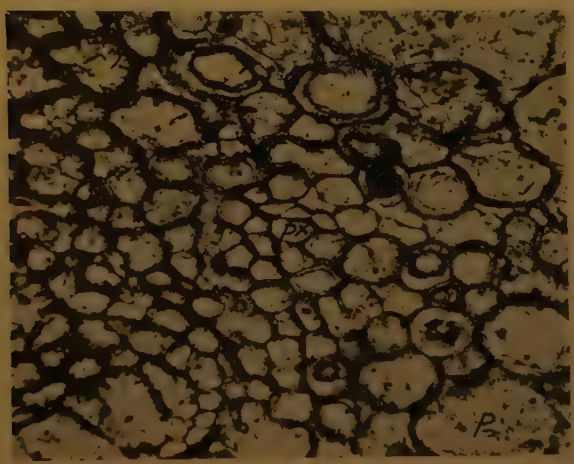
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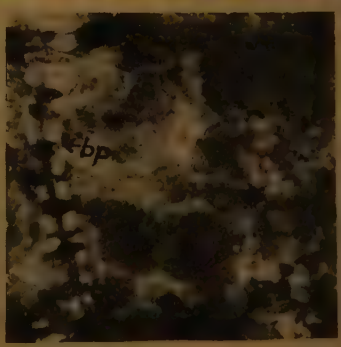
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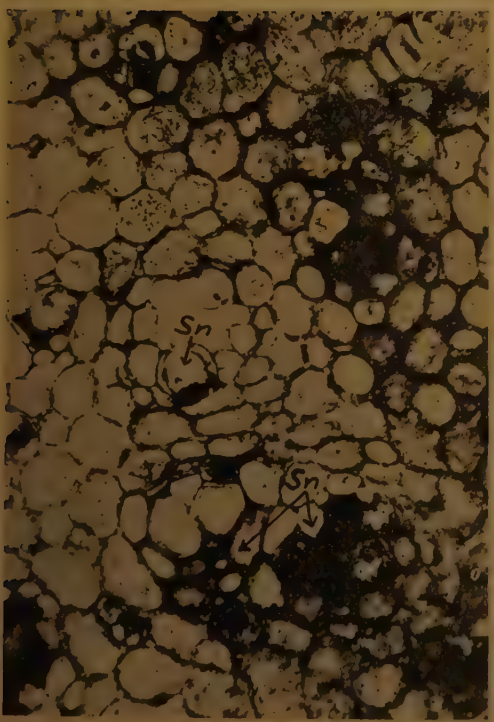

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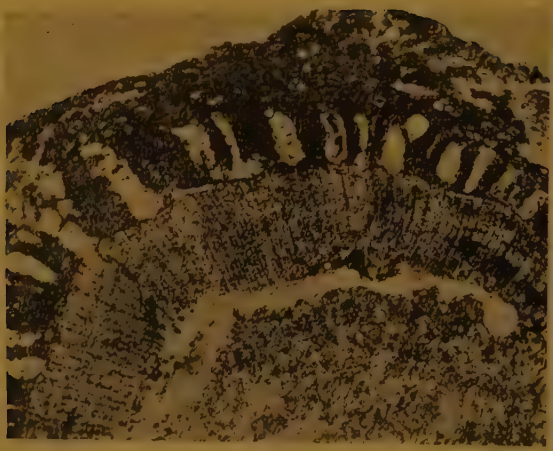

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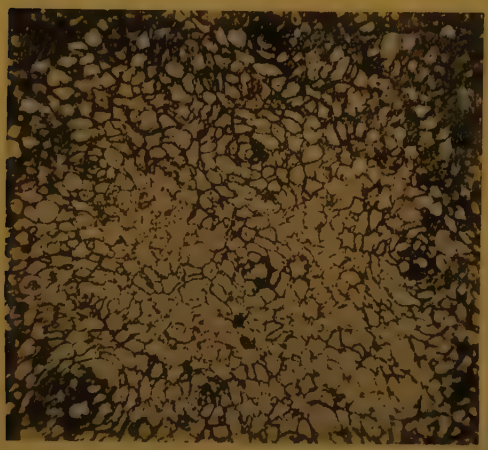

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ENZYMIC STUDY OF CERTAIN PARASITIC FUNGI

By V. P. Damle

Pratap College, Amalner, E. K.

(Received for publication on December 5, 1951)

Introduction

This work consists mainly of an enzymological and a plant-pathological comparison of a number of Pythium isolates. To a certain extent the parallel behaviour of *Botrytis cinerea* has also been studied. The investigation constitutes a part of the "Studies in the Physiology of Parasitism" which have been carried on at the Imperial College of Science and Technology, London, for some years.

A main line of study in the series of papers just referred to has been to see how far the capacity of an organism to produce the tissue-rotting enzyme pectinase is correlated with the pathogenic capacity of that organism. This had led to an examination of the effect of cultural conditions on enzyme secretion and to a study of the detailed properties of the enzyme so produced. This is the topic of the present paper.

Previous Work

It will be convenient to divide this into two parts, the first enzymological and the second plant-pathological.

Literature on Pythium Enzyme.—Outside the work done in the Imperial College Laboratory, London, there has been very little on this subject. Hawkins and Harvey (1919) in U.S.A. studied the mode in which hyphæ of *Pythium debaryanum* penetrated the cell walls of potato tubers, but they considered this to be essentially a mechanical process, so that they paid little attention to any cell wall attacking enzyme. Chona (1932), Menon (1934) and Fernando (1937), all working at the Imperial College, made preparations of pectinase enzyme from Pythium by extracting the parasitised tissue of living surface-sterilised or of autoclaved potatoes. In this way they obtained quite active preparations, but they were unable to obtain these from cultures on such artificial media as they had tested in this connection. The detailed properties of Pythium enzyme, auch as optimal P_H and relative activity on plant tissue under varied conditions, were not identical with those of the enzyme of *Botrytis cinerea*. These comparisons were made between enzymes prepared under different conditions, *viz.*, Pythium enzyme extracted from parasitised potato tissue, Botrytis enzyme prepared on a variety of artificial media (since this fungus normally does not attack potato). It was obviously desirable to determine whether active preparations of both fungi could be obtained

from the same medium, and this meant the finding of an artificial medium suitable for production of Pythium enzyme.

Ashour (1948), working at the Imperial College, obtained a medium which was satisfactory in this respect. He found, however, a very pronounced optimum in the activity of enzymic secretion at a certain concentration. Either above or below this, enzymic activity fell off sharply. This suggested that Pythium was rather selective in its nutritive requirements for free enzymic secretion, whence the failure of the other workers mentioned to obtain satisfactory preparations from a number of ordinary media. This finding was the starting point of the research carried out by the writer.

Recently Brandenburg (1948, 1950), has described toxic effects on plant tissues which are brought about by culture extracts of a pythium of the *irregulare* type. He makes no mention of macerating effects. The properties of the active substance were studied in considerable detail, but apart from the statement that it was thermolabile, there is nothing in Bradenburg's account to enable one to say whether he was dealing with the tissue-macerating enzyme or with some other substance. The relation of his work to that of the writer is thus not clear at the moment.

Literature on Pathogenicity of Pythium.—There are numerous statements in the literature regarding Pythium species as parasites, chiefly in connection with damping off (pre- and post-emergence) of seedlings. As the plant-pathological section of this paper is confined to potato and lettuce as hosts there is no need to refer to the wider literature.

The occurrence of Pythium on potato tubers, in which it causes a soft watery rot usually under storage conditions, has been reported from a number of countries. Thus Hawkins (1916) describes a "watery wound rot" of potatoes in California caused by *P. debaryanum*. A similar disease has been described from Australia, Cyprus and other countries. In the summarised periodic reports of the last eight years (from 1943 up-to-date) of the Ministry of Agriculture, Harpenden, loss of potatoes in clamps due to watery wound rot has been recorded from various parts of England and Wales and in almost all these cases the causal organism is stated to be *P. ultimum*.

On lettuce it has been known for a long time as one of the agents of damping off. Its occurrence in the field was described in some detail by Abdel Salam (1933) and its general relation to cropping of lettuce in the area of Slough indicated by Smieton and Brown (1940). The relation of soil moisture and temperature to the extent of disease has been studied and the efficiency of seed dressings as control measures tested by Sidky (1947), Tolba (1949), Jacks (1951) and others. Apart from serious loss which may occur in the pre-emergence stage of the seedlings, it is stated that as a disease of the young growing crop, attack by Pythium is much less formidable than that by *Rhizoctonia solani*.

Materials and Methods

The fungal cultures used in this work comprise the following:—

(1) *Pythium debaryanum*, isolated from lettuce, stock laboratory culture.

(2) *Pythium debaryanum*, isolated from tomato, stock laboratory culture.

(3) *Pythium ultimum*, isolated from lettuce, from Commonwealth Mycological Institute.

(4) *Pythium intermedium*, isolated from Kentia, from Commonwealth Mycological Institute.

(5) *Pythium intermedium*, isolated from hops, from Commonwealth Mycological Institute.

(6–9) Isolates of Pythium made by the writer. These will be described and named in the next section.

(10) *Botrytis cinerea*, isolated from lettuce, stock laboratory culture.

Stock cultures of the above were maintained on tubes of oatmeal agar (4% oatmeal, 1·5% agar) or potato dextrose agar (20% peeled potato, 2% dextrose, 1·5% agar). These were started at 25° C. for three days, after which they were kept at laboratory temperature. Growth was more luxuriant on the latter medium.

A considerable part of the work to be described concerns the capacity of the isolates to produce pectinase enzyme. For preparing this, medicine bottles of 250 c.c. capacity were used. Into these 25 c.c. of medium, either as synthetic solution or as natural decoction, was placed and sterilised. These bottles are fitted with a loose screw cap and it was found that such caps conveniently replace cotton wool plugs, being much quicker to manipulate and quite efficient in preserving sterility. After sterilisation the bottles were seeded when cool.

The media used were:—

(*a*) *Potato decoction.*—This was made at two different strengths. Potato tissue was steamed in water for 1 hour, pressed through muslin, the coarser material being rejected. The liquid portion corresponding to 20 gm. potato was made up to 100 c.c. with water to give the weaker medium. For the stronger medium 50 gm. potato was used.

(*b*) *Lettuce decoction.*—5 gm. of dried lettuce leaves or 50–75 gm. of fresh leaves were boiled for 1 hour. The coarser material strained off and the liquid made up to 100 c.c. This was used at various dilutions.

(*c*) *Synthetic medium.*—The basal medium was that used by Ashour (1948), with the following composition:

Glucose	5 gm.
Starch	25 „
Asparagin	4·5 „
Peptone (Gurr's bacteriological grade)	4·5 „
K_3PO_4	3·4 „
$MgSO_4$	1·9 „
Distilled water	1 litre

As will appear in a later section, the properties of this medium from the point of view of enzyme production vary according to the exact manner in which the medium is made up and sterilised.

The bottles, prepared as just described, were seeded with various isolates. For *Botrytis cinerea*, 1·5 c.c. of a dense spore suspension (approx. 10^7 spores per c.c.), taken from a 15 to 20-day old culture on potato agar (20% potato agar), was added. For the Pythium isolates, spores were not freely available so that mycelial inoculum were used. Discs were cut with a sterilised No. 2 cork borer (5 mm. diam.) from the edge of 2-day old cultures on plain agar,* and these were dropped into the bottle, six into each. Later it was found more convenient to cut, by means of a stencil, a marginal strip of area equal to that of six discs. This was then cut into about 15–20 small pieces and these inserted into the bottle. In addition to being more convenient, this method has the advantage of giving more foci of inoculum and therefore of tending to approach the more uniform conditions obtained by a seeding of spores.

After inoculation the bottles were arranged on their flat side during incubation at 20–25° C. This presented a shallow layer so that good aeration was ensured throughout the medium and also made available a larger surface for growth of the fungus. Incubation period ranged from 5–10 days.

At the end of the incubation period the fungal mats were removed, washed in running water for quarter of an hour and their volumes measured by centrifugation in graduated tubes for ten minutes at 2,500 R.P.M. Alternatively they were dried in weighed filter-papers at 100° C. and their dry weight determined on a Jolie spring balance. The weights are recorded in Jolie units (14 units on the scale being equivalent to 1 gm.).

Enzymic studies were limited to the excreted enzyme, *i.e.*, exo-enzyme. The filtrates from the bottles were used in the crude form, *i.e.*, without any purification, as it was found that even with a single precipitation with acetone a considerable amount of enzyme was lost.

Pythium enzyme was also obtained from potato tissue (var. King Edward) parasitised by the fungus. For this purpose tubers were surface sterilised with 0·1% mercuric chloride or methylated spirit

* In this connection only plain agar cultures were used to minimise the complication arising from the introduction of extraneous nutritive substances.

and cavities cut in them with a No. 2 cork borer to a depth of 0·5–1 cm. These were inoculated with a disc of Pythium mycelium on plain agar of the same diameter and the wound sealed with melted paraffin wax, control tubers had discs of plain agar. Incubation was at 20–25° C. for 3–5 days. All the control tubers remained sound whereas active rotting developed with the Pythium inocula. The rotted tissue was scraped out and filtered through muslin, after which it was centrifuged to remove debris. A bulk of this material (*e.g.*, 3–4 litres) was built up and kept in presence of toluene at 4° C. In this form or when purified it maintains its activity for over 10 months and this enabled it to be used as a standard of comparison from time to time. When required it was either used as such or it was purified by 1–3 precipitations with acetone.

Pectinase activity was determined by the tissue-disc method. For this purpose cylinders were cut with a No. 11 cork borer (16 mm. diameter) from potato tubers (var. King Edward) or turnip, or with a No. 5 cork borer (8 mm. diameter) from cucumber fruit, injected with water under reduced pressure and cut on a hand microtome into discs of 0·5 mm. thickness. As far as possible the discs were taken only from the intravascular portion of each cylinder. They were thoroughly washed in distilled water, pressed on blotting paper and three of them were placed in 3 c.c. of the enzymic preparations to be tested. The average time for loss of coherence to take place was noted. This reaction time (R.T.) is inversely proportional to the activity of the preparation. As it will be noted later the number of determinations of pectinase activity were also made by use of a viscosimetric method.

Throughout this work determinations of P_H values were made with the aid of the Universal Buffer Solutions prepared by British Drug Houses Limited, according to the Colour Chart of Indicators made for the determination of hydrogen ions by Mansfield Clark. The determinations were checked from time to time on a Cambridge portable type P_H meter.

Pathogenicity tests of Pythium isolates were carried out with potato tubers and also with lettuce seeds and seedlings.

Potato tubers (var. King Edward) were set up in the manner already described for the preparation of Pythium enzyme (p. 16) and the amount of attack determined by weighing the rotted tissue after a given time. Alternatively the same kind of test was carried out on cylinders of tissue cut with a No. 11 cork borer. The latter were placed in moist specimen tubes and inoculated at the top with a disc of Pythium mycelium. Autoclaved tubers or cylinders were also used for comparison.

The work with lettuce was carried out as follows:—

Seed of lettuce (var. Imperial) was surface sterilised by 5 minutes immersion in 0·1% mercuric chloride solution or 1% bromine water, followed by 5 washings with sterile water in the case of the former. The bromine method was found to be equally efficient and to be simple and was therefore adopted in all the later work. After

immersion the seeds were merely pressed between sterile filter-papers and left for 5–6 hours for the residual bromine to volatilise. They were then ready for use. From such treated seed, sterile seedlings were raised in Petri dishes at 20–25° C. on sterile wet filter-paper or on plain agar.

Seed or seedlings so prepared were used in pathogenicity tests either by placing them on plain agar cultures of the various Pythium isolates or sowing (or planting) in soil or sand containing Pythium inoculum. For the latter purpose the standard cornmeal technique was adopted. A 2% mixture of cornmeal in sand was adjusted to approximately 25% water content, autoclaved and inoculated with the various Pythium isolates and incubated for 15–20 days at temperatures optimal for the isolates under consideration. This culture was then added in the proportion 1 : 20 to sterilised sand or soil in glass capsules, adjusted to 40–50% water content and incubated for 8 days. Seed or seedlings were then placed in the capsules.

Cultural Comparison of Isolates

During July and early August 1950 isolations were made from the soil of plots at Slough Experimental Station which were known to be contaminated with organisms causing damping off of lettuce. For this purpose soil samples were brought to the laboratory, filled into sterilised glass capsules, watered with sterile water, seeded or planted with sterilised lettuce (var. Imperial), covered with glass plates and left at green house temperature (15–20° C.). In about four to six days the contents of the capsules were examined for damping off which took the form of failure to emerge on the part of some seeds or of root attack of others and of the seedlings which were transplanted. Affected seeds or seedlings were washed under the tap, then rinsed with sterile distilled water or were dipped in diluted bromine water for two minutes, washed in sterile distilled water, placed between sterile filter-papers to minimise bacterial contamination and finally plated on acidified agar plates (1·5–2% agar adjusted to P_H 5–5·5) and incubated at 20–25° C. As an acidity of P_H 5 or thereabout materially checks Pythium growth, an alternative method was used and found to give better results. This was to plate the washed material on plain agar and to take hyphal tip transfers from these, more especially from below the surface of the agar. This was done by inverting the agar film into the lid of the Petri dish.

In addition to a number of cultures of Fusarium and Rhizoctonia, 27 isolates of Pythium were obtained. These could be grouped into the following species, according to the classification of Matthews (1931) and Middleton (1944):—

P. debaryanum:—11 Isolates; characterised by the presence of diclinous antheridia on long stalks, one to three—more often two—per oogonium; Sporangia borne singly; mycelium stout, branching in racemose fashion; growth on plain agar distinctly visible when viewed in transmitted light (Fig. 1).

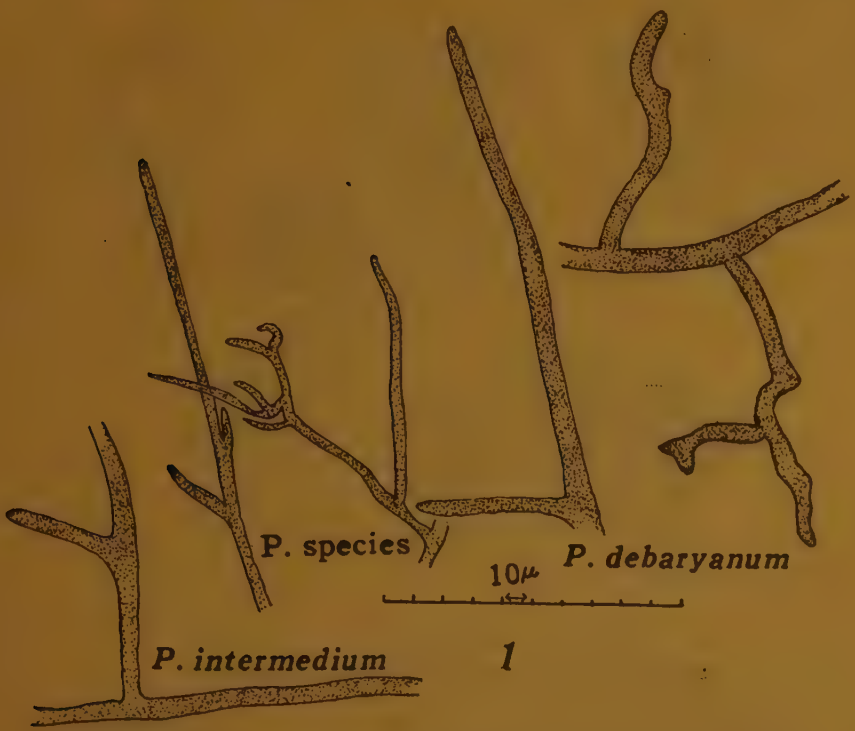

FIG. 1. Hyphæ of three species of Pythium, showing the difference in their habit.

P. ultimum:—7 Isolates; single monoclinous antheridium per oogonium, arising just below the latter and sharply curving to become attached to the oogonial wall; Sporangia borne singly; mycelium and macroscopic appearance as in *P. debaryanum.*

P. intermedium:—5 Isolates; sexual organs not formed; Sporangia catenulate; mycelium less stout than in the two preceding but culture clearly visible macroscopically (Fig. 1).

P. species:—4 Isolates; characterised by spiny oogonia; Sporangia borne singly; hyphæ intricately branched and very fine so that culture on plain agar is transparent to the naked eye. Sufficient sporangia were not available to allow of exact specific determination, but this isolate appeared to agree most closely with *P. mastophorum* and *P. polymastum* of Middleton. In the absence of a clear identification it will be referred to merely as P. species (Fig. 1).

There were no obvious morphological differences between the various members of each species group, any differences that showed, as for example in number of antheridia per oogonium, being seen in different parts of the same culture. One isolate from each group was

FIG. 1 a. *Pythium debaryanum*.—(Isolate No. 6). *a–a*, Sexual apparatus. *a-b*, *c*, Oogonia with two diclinous antheridia. *d*, Oogonium with single monoclinus antheridium. Oogonia contain aplorotic, smooth oospore. *e* and *f*, Sporangia.

therefore selected for more detailed study. The list given on p 15 may now be completed as follows:—

(6) *Pythium debaryanum*
(7) *P. ultimum*
(8) *P. intermedium*
(9) P. species

} All isolated by the writer from Slough soil

A fuller description of the four groups of isolates is the following:

(6) *P. debaryanum*:—Hyphæ 6–8 μ in diam.; Sporangia spherical, terminal or intercalary, 23–26 μ; Oogonia smooth, spherical, mostly terminal, 22–27 μ—usually 25 μ in diam.; Antheridia 1–3 per oogonium, generally diclinous, though monoclinous antheridia are not uncommon, 8 μ; Oospores smooth, aplerotic, 18–25 μ, average 22 μ (Fig. 1 a).

(7) *P. ultimum*:—Hyphæ 6 μ in diam.; Sporangia spherical, terminal or intercalary, 23–26 μ; Oogonia smooth, spherical, terminal 23–27 μ—usually 23 μ in diam.; antheridia one per oogonium, monoclinous, swollen, hypogynous, arising immediately below the oogonium and sharply curving towards the oogonial wall, 10 μ; Oospores smooth, aplerotic, 16–22 μ, average 20 μ (Fig. 2).

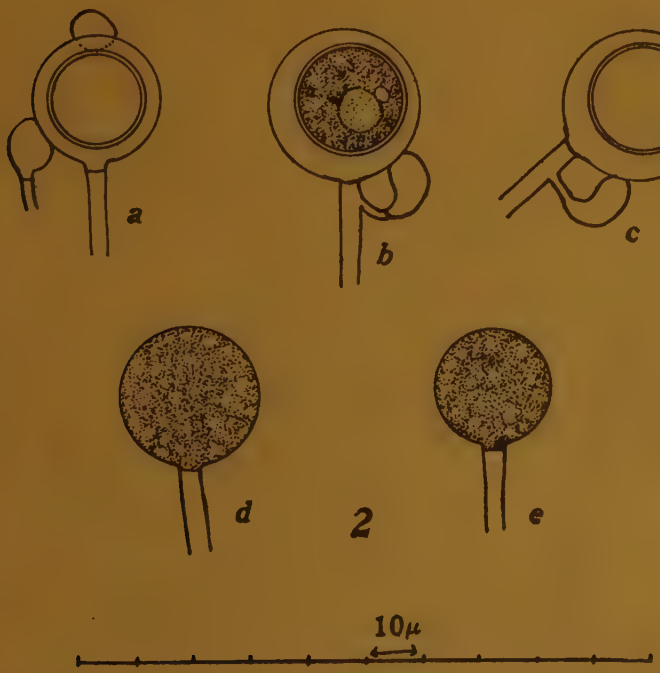

FIG. 2. *Pythium ultimum* (Isolate No. 7).— *a–c*, Sexual apparatus. *a*, Oogonium with two diclinous antheridia. *b* and *c*, Oogonia with single monoclinous antheridia. *d* and *e*, Sporangia.

(8) *P. intermedium:*—Hyphæ 5–6 μ; Sporangia spherical or subspherical, catenulate, 16–25 μ—usually 18 μ; Sexual organs not formed (Fig. 3).

(9) P. speciss:—Hyphæ 3–5 μ; Sporangia 18–20 μ; Oogonia spherical, smooth when young, echinulate when mature, 22–25 μ, average 22 μ without spines, 30–32 μ with spines (occasional large specimens were 30 μ without, 42 μ with spines), very few antheridia observed; Oospores smooth, spherical, aplerotic, 18–22 μ (Fig. 4).

The growth rates and temperature responses of these Pythium isolates (6–9) were studied. For this purpose plain agar plates were inoculated at the centre with discs (5 mm.) of cultures on the same medium and incubated at temperatures 4, 10, 15, 20, 25, 30 and 35° C. The increase in diameter of the colonies was measured every 24 hours till the cultures completely filled 9 cm. Petri dish. In a few such tests the inoculated dishes were kept at 20° C. for a day, the growth of the colony marked on the glass and then they were transferred to the various temperatures. The latter method gave more uniform results but did not show any material difference in the growth rates as such. The mean measurements of diameters of three

FIG. 3. *Pythium intermedium* (Isolate No. 8). — *a*, Catenulate sporangia. *b*, Germinating sporangium.

colonies after two days' incubation at the various temperatures are given in Fig. 5 and the growth rates at 25° C. are recorded in Fig. 6.

It will be seen from Fig. 5 that the curves for *P. debaryanum* (No. 6) and *P. ultimum* (No. 7) are identical with an optimum between 25° C. and 30° C. The curve for P. species (No. 9) has the same shape but it grows somewhat more slowly. These three curves fall sharply between 30° C. and 35° C. and cultures kept at 35° C. for two days were found to be dead as they did not grow when transferred after that period to 25° C. *P. intermedium* (No. 8) has an optimum between 20° C. and 25° C. and it was killed by two days' exposure to 30° C.

Fig. 6 brings out what is the main difference in growth between the various isolates. After about a day all the cultures show much the same growth rates, the curves then running parallel. The isolates

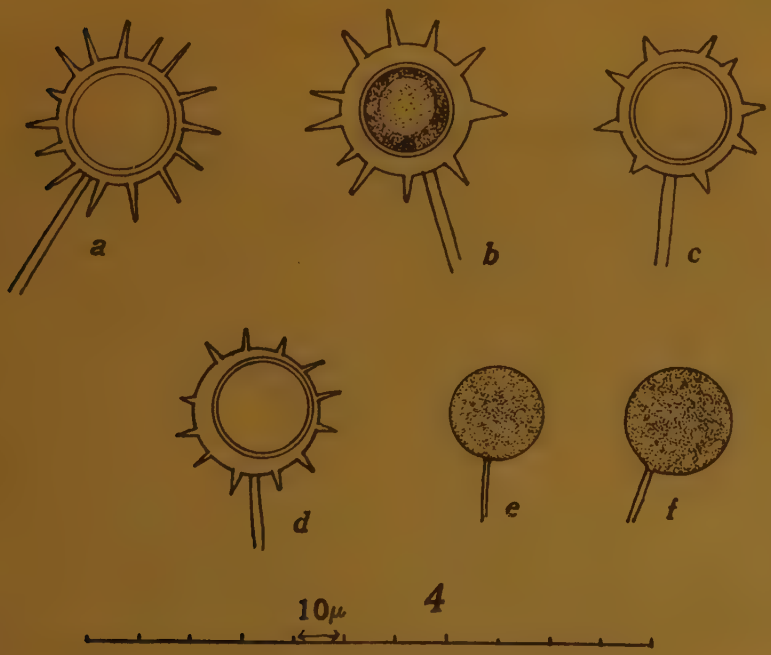

FIG. 4. *Pythium species* (Isolate No. 9). — *a–d*, Echinulate oogonia, each containing an aplorotic, smooth oospore. *e* and *f*, sporangia.

vary considerably in their initial rates of growth, there being a distinct lag in starting with No. 8 and a less pronounced one with No. 9.

It may be stated here that in respect to their measurements of fruiting bodies and growth and temperature effects Nos. 1, 2 and 3 of the list on p. 15 agreed with Nos. 6 and 7 and No. 5 with No. 8. No. 4, which is also of intermedium type definitely grew more slowly (2 cm. as against 3 cm. per day of No. 8).

A striking feature of the cultural behaviour of the isolates of Pythium listed above is the distinct antagonism of No. 9 (P. species) towards all the others. When any two of the latter are placed on a plain agar plate at opposite diametric points and incubated at 20–25° C. the cultures grow into each other without any suggestion of mutual interference and if they are isolates with characteristic sporing structures—*e.g.*, if the pair be *P. debaryanum* and *P. intermedium*—the fact of the interpenetration is shown by the presence of the fruiting structures of both over the whole plate. When, however, any one of these isolates is coupled with No. 9, they grow in the normal manner until they meet, but there is no further advance of the former, whereas No. 9 grows across to the opposite side, as can be shown by the presence of spiny oogonia over the whole plate. The effect is clearly visible to the naked eye because of the arrested growth of the coarser hyphæ in the middle of the plate, the

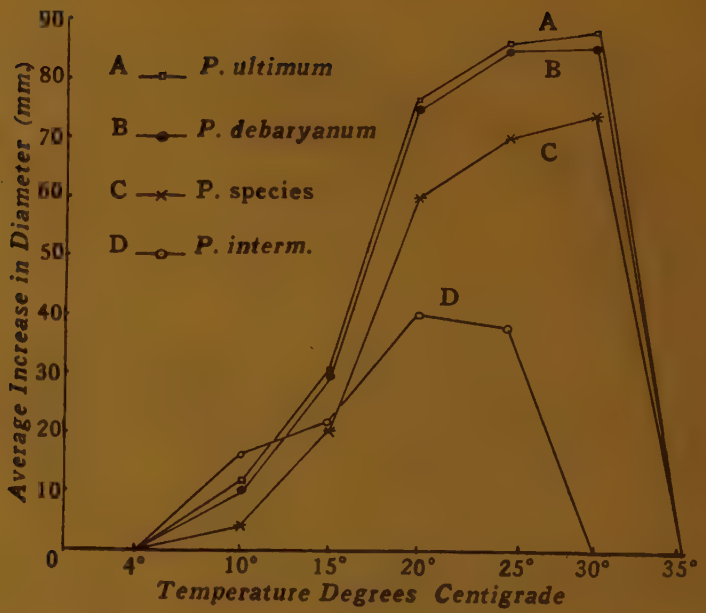

FIG. 5. Effect of temperature on the growth of four species of Pythium (recorded after two days' incubation of plain agar cultures).

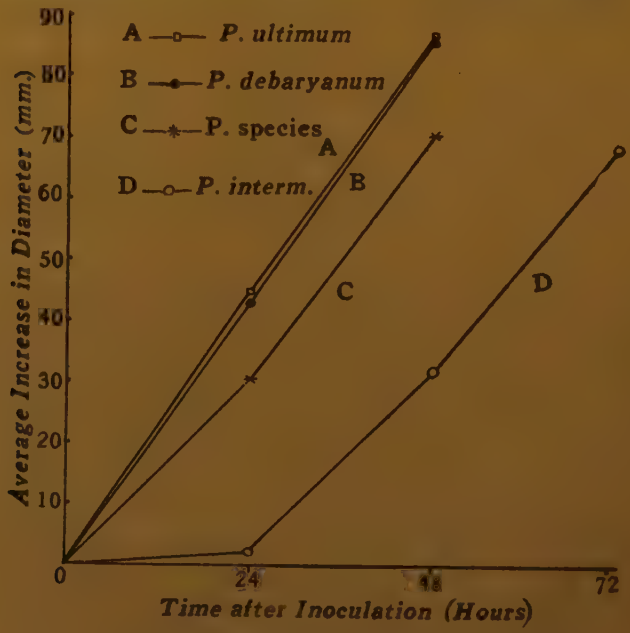

FIG. 6. Growth rates of four species of Pythium (Plain agar cultures incubated at 25° C.)

half solely occupied by No. 9 being almost transparent, probably by reason of its very fine hyphæ (Fig. 7). This position was unaltered even after two months.

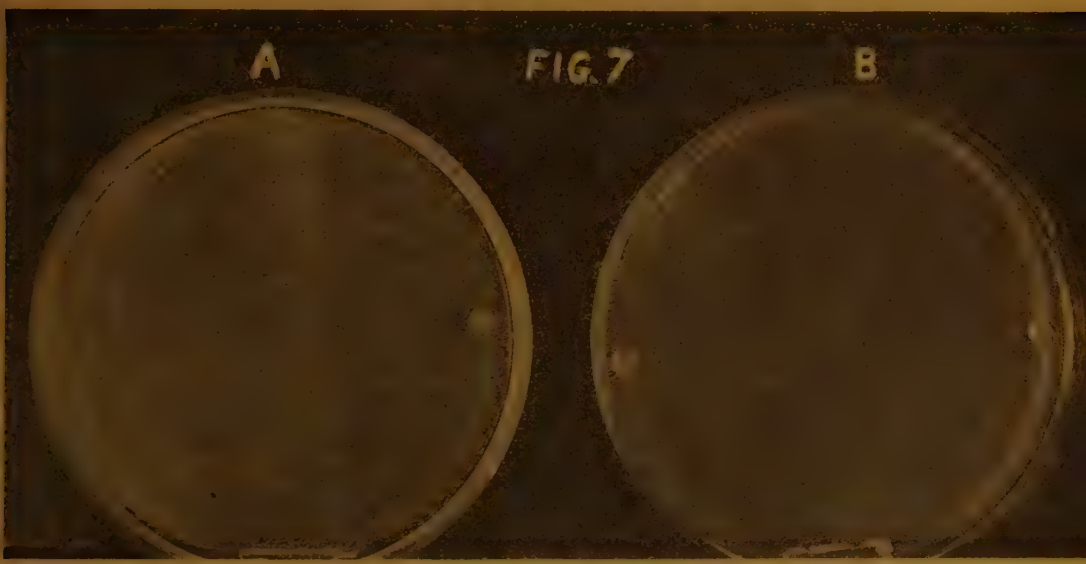

FIG. 7. Antagonism between species of Pythium. A, Dual culture of *Pythium debaryanum* (No. 2) and Pythium species (No. 9). B, Dual culture of *Pythium intermedium* (No. 8) and Pythium species (No. 9). After ten days incubation. Note the arrested growth of the coarser hyphæ in the direction of the inoculum of P. species in A and B; the transparent part of both plates being completely occupied by the fine hyphæ of P. species (No. 9).

Further evidence of the antagonistic effect of No. 9 on the other Pythium isolates is seen by placing a mixed inoculum (No. 9 + any other) at the centre of a plain agar plate. The culture which grows out consists mainly of No. 9, the other partner being represented by isolated strands.

An experiment was conducted in which isolates No. 9 was grown for 10 days in 25 c.c. of S medium. The culture solution was then passed through a sterile Seitz filter and placed in a central circular cavity made on a plain agar plate. A disc of isolate No. 2 (*P. debaryanum*) was placed at the centre of the plate and its growth compared with that on a similar plain agar plate having a central cavity filled with sterile distilled water. The culture on the former required 3–4 days to reach the edge of the plate as against 1·5–2 days required by the latter. Though this experiment was merely of a preliminary nature, its results fall into line with those already described.

This antagonistic effect was also demonstrated in the pathogenic behaviour of the isolates, as will be pointed out a little later.

A similar inhibitory interaction between the species of Pythium, when they are grown together in dual cultures on plain agar, has been reported by Drechsler (1943).

Effect of Medium on Pectinase Secretion

1. *Method of Preparation*

In the first attempts of the writer to prepare enzymic extracts of Pythium, Ashour's strain of *Pythium debaryanum* (No. 1 of the list on p. 15) and his standard medium (which will be referred to as S medium henceforth) were used. In confirmity with the practice adopted in the Imperial College Laboratory when synthetic media are being made, the S medium was sterilised in two portions, *viz.*, the phosphate separate from the rest; thus instead of adding all the constituents listed on p. 17 to 1 litre of water, the phosphate was dissolved in, say, 200 c.c. the remaining constituents in 800 c.c. and these portions autoclaved separately. They were then combined, just before inoculation, when they had somewhat cooled. The purpose of this was to prevent the copious precipitation of magnesium phosphate. In Ashour's work all the constituents were autoclaved together. A further difference was that the volume of medium in each bottle was increased from 10 c.c. to 25 c.c. In all other respects, the details of Ashour's method of preparation were followed.

The extracts obtained in this way were disappointingly weak, giving R.T. of about 150–180 minutes as against 30–50 minutes found by Ashour. As the difference might have arisen from the greater amount of medium used per bottle, the amount of inoculum was increased, from one disc of agar culture to six such discs. This was an improvement, inasmuch as it reduced the R.T. to about 90 minutes, which still represented a distinctly weaker solution than that obtained by Ashour. Inoculation with more than six culture discs had no further beneficial effect. However, this increase in the amount of inoculum per bottle reduced the period of incubation from 10 days to 5 days.

Further attempts to prepare stronger extracts were made by varying the total concentration of the medium, using 2S, S, S/2 and S/5, but with no success.

In view of the possibility that Ashour's original culture had degenerated the experiments were repeated with one more recently isolated culture—No. 2 of p. 15—but this also gave no improvement.

The trouble was finally tracked down to the effect of phosphate when autoclaved in presence of the carbohydrate constituent of the medium. This effect will now be illustrated.

Medium S was made up in three different ways as follows:—

(*a*) all the constituents mixed before autoclaving,

(*b*) phosphate autoclaved separately from the rest, and combined when cooled,

(*c*) sulphate autoclaved separately from the rest, and combined when cooled,

Cultures in medicine bottles were set up in the standard manner on the above three media and they were incubated at 25° C. for 5 days. Results are given in Table I.

TABLE I

Effect of Method of Preparation of Medium

Media	Initial ρ_H	Final ρ_H	Dry wt. mycel. in units	R. T. minutes
(*a*)	7·4–7·6	7·8–8	3·2–3·4	60–65
(*b*)	7·6–7·8	8–8·2	3·2	85–90
(*c*)	,,	7·8–8	3·5	30–35

Medium (*a*) represents Ashour's method ; medium (*b*), the method which had hitherto been used by the writer.

The results of Table I clearly point out that the autoclaving of phosphate with some other constituent of the medium has a beneficial effect on enzyme secretion. The intermediate result obtained by Ashour would presumably be due to the presence of phosphate during autoclaving but at a lower concentration than in medium (*c*) because of its partial precipitation by the magnesium sulphate present.

In order to determine which constituent or constituents were affected by being autoclaved in the presence of phosphate, the latter was autoclaved in turn with each constituent of medium S and added afterwards to the rest. The following were the combinations tested:—

(*a*) phosphate with all other constituents except $MgSO_4$,
(*b*) sulphate with all other constituents except K_3PO_4,
(*c*) phosphate and glucose,
(*d*) phosphate and starch,
(*e*) phosphate and asparagin,
(*f*) phosphate and peptone,
(*g*) phosphate by itself, glucose by itself.

The results are given in Table II.

The results show that the requirement for the preparation of an active enzymic extract is that the phosphate should be autoclaved with the carbohydrate. That some chemical change takes place when these specific constituents are autoclaved together is indicated by the development of a brown-yellow colour in the medium, an effect which is not shown by any other combination.

It was interesting at this stage to test the effect of the new recipe of the preparation of the medium on the culture of Pythium (No. 1) discarded because of its supposed degenerated condition. Media were made by autoclaving phosphate with (*a*), and separately, from carbohydrate (*b*) and inoculated with isolate Nos. 1 and 2 and incubated

TABLE II

Effect of Autoclaving Phosphate with Individual Constituents

Media	Colour	Initial P_H	Final P_H	R. T. minutes
(*a*)	Brown yellow	7·6–7·8	7·8–8	30–35
(*b*)	Colourless	8–8·2	,,	75–80
(*c*)	Brown yellow	7·8–8	,,	40
(*d*)	,,	,,	,,	40–45
(*e*)	Colourless	,,	,,	80–85
(*f*)	,,	,,	,,	90
(*g*)	,,	,,	,,	90–100

in the usual way. The results of the experiment after five days' incubation are given in Table III.

TABLE III

Comparison of Enzymic Activity of two Isolates

	Isolate No. 1				Isolate No. 2		
Media	Initial P_H	Final P_H	Vol. mycel. c.c.	R. T. min.	Final P_H	Vol. mycel. c.c.	R. T. min.
(*a*)	7·6–7·8	7·6	3·1	35	7·8	3·5	30
(*b*)	8–8·2	7·8	3·4	100	7·8	3·3	90

It will be seen from Table III that the first culture behaved exactly in the same way as culture No. 2. In the later work, however, *Pythium debaryanum* (No. 2) was consistently used and unless otherwise specified S medium was made by autoclaving phosphate with the rest of the medium except $MgSO_4$ and adding the latter to it after cooling.

The phosphate of the medium S is the tribasic one, which is definitely alkaline in reaction. The question therefore arose whether the effect under consideration was due to the phosphate as such or merely to its alkalinity. This was determined by experiments along two lines.

In one of these all the constituents of medium S except phosphate, *viz.*, glucose, starch, asparagin, peptone and magnesium sulphate were autoclaved at two P_H levels and after that combined with separately autoclaved phosphate on cooling. The following three media were prepared :—

(*a*) $MgSO_4$ + rest of Medium S,
(*b*) K_3PO_4 + rest of Medium S at P_H 7·2–7·4,
(*c*) K_3PO_4 + rest of Medium S at P_H 8·6–8·8.

The + sign indicates that the constituent on either side were autoclaved separately before being combined. The media so prepared were inoculated in the usual manner with Pythium isolate (No. 2) and after 5 days' incubation at 25° C., they gave enzymic preparations with R.T.s shown in Table IV.

TABLE IV

Effect of P_H *on Enzyme Secretion*

Media	Colour	Initial P_H	Final P_H	Vol. mycel. c.c.	R.T. min.
(*a*)	Brown yellow	7·8–8	7·4–7·6	3·7	30–35
(*b*)	Colourless	8–8·2	,,	3·4	65–70
(*c*)	,,	8·6–8·8	7·8–8	3·6	60–70

The initial P_H's of the three media showed a variation of about 1 P_H unit, but all reached the same slightly alkaline (7·4–8) point after 5 days' growth. These small P_H differences had no material effect on fungal growth on the various media; nevertheless the enzymic extracts obtained from (*b*) and (*c*), *i.e.*, from the media in which phosphate was autoclaved separately from the other constituents, were definitely weaker than that obtained from the standard medium (*a*).

In the second set of experiments the effect of the three potassium phosphates on enzyme secretion was studied. For this purpose the following media were made up:—

(*a*) $MgSO_4$ + rest of medium S,
(*b*) K_3PO_4 and glucose (9·3; 7·9)* + rest of medium S,
(*c*) K_3PO_4 + rest of medium S,
(*d*) K_2HPO_4 and glucose (8·3; 7·6) + rest of medium S,
(*e*) K_2HPO_4 + rest of medium S,
(*f*) KH_2PO_4 and glucose (4·9; 5·9) + rest of medium S,
(*g*) KH_2PO_4 and glucose and N/10 NaOH to give P_H 8·3 (8·3; 8·2) + rest of medium S,
(*h*) KH_2PO_4 + rest of medium S.

* These figures denote the P_H before and after autoclaving.

The results are set out in Table V.

TABLE V

Effect of Phosphates on Enzyme Secretion

Media	Colour	Initial P_H	Final P_H	Dry wt. mycel. in units	R. T. min.
(*a*)	Brown yellow	7·6–7·8	7·8–8	3·5	35
(*b*)	,,	,,	7·6–7·8	3·5–4·3	35–40
(*c*)	Colourless	8·2	7·4–7·8	3–4·1	120–130
(*d*)	Yellow	7·0	7·6–7·8	2·2–3	50
(*e*)	Colourless	7·4	,,	1·8–2·5	140
(*f*)	,,	5·6	5·6–5·8	1·5–2·5	>150
(*f'*)			adjusted to 7·8		>150
(*g*)	Yellow	8·2	7·9–8	2·8–3·2	50
(*h*)	Colourless	5·6	6·2–6·4	1·3–1·9	>150

The following conclusions arise from Table V:—

(1) Comparison of media (*a*), (*b*) and (*c*) shows that active enzymic preparation is obtained when alkaline phosphate is autoclaved with all the constituents, except $MgSO_4$, or when it is autoclaved with glucose alone.

(2) Media (*d*) and (*e*) which correspond respectively with (*b*) and (*c*), except for having the dibasic phosphate, are nearer the neutral point at the start of the experiment, but finally reach the same P_H as (*b*) and (*c*). The amount of growth produced is somewhat less; fairly good enzymic activity is shown by (*d*), weak activity by (*e*).

(3) For media prepared with monobasic phosphate (*f*–*h*), growth is rather poor and enzymic activity very low if the medium is used without further adjustment, *i.e.*, as in (*f*) and (*h*). Both these media are acid throughout, and therefore unfavourable for growth of Pythium. Also the final P_H is unsuitable for the action of any pectinase present, the optimum for which lies between P_H 7·6–8·6. The preparation (*f'*) differed from (*f*) merely by having the final P_H adjusted to 7·6–7·8, *i.e.*, to the optimum for the enzyme. Nevertheless activity was very low, indicating that very little enzyme had been produced. Medium (*g*) is almost equivalent to (*b*) and shows much the same enzymic activity. The summarised result of Table V is that good enzymic activity is obtained when glucose is autoclaved with the tribasic or dibasic but not with monobasic (acid) phosphate.

The overall conclusion from the results of Tables II–V is that it is not alkali alone nor phosphate independently, but it is phosphate under alkaline conditions when autoclaved with the carbohydrate which makes the medium favourable for the fungus to secrete active enzyme.

The effect just described for glucose is also shown by fructose. Table VI gives the results of a comparison of media, based on Medium S, in first six (*a–f*) of which the sugar was glucose, in the last four the same quantity of fructose. In several of these media, *viz.*, (*b–d*) and (*h–j*), the constituents were autoclaved in three separate batches, as indicated by two + signs, and the effect of autoclaving the sugars at different P_H levels was also tested.

TABLE VI

Comparison of Glucose and Fructose for Enzyme Production

Media	Initial P_H	Final P_H	Dry wt. in units	R. T. min.
(*a*) Glucose & K_3PO_4 + rest of (9·4; 7·6)* medium	7·6	7·8	3·6	35–40
(*b*) Glucose + K_3PO_4 + „ (5·5; 4·8)	7·8	8·0	2·5–3·1	60–65
(*c*) Glucose + „ + „ (6·0; 5·2)	„	„	2·3–2·5	60
(*d*) Glucose + „ + „ (9·0; 5·0)	„	„	2·8–3·0	55–60
(*e*) K_3PO_4 + „	8·2	7·8	3·0–3·4	80–90
(*f*) Whole S medium autoclaved together	7·6	„	3·2–3·4	60–65
(*g*) Fructose & K_3PO_4 + rest of (9·4; 7·6) medium	„	„	3·7	35
(*h*) Fructose + K_3PO_4 + „ (5·5; 5·2)	7·8	8·0	2·5–2·6	50
(*i*) Fructose + „ + „ (6·0; 5·2)	„	8·2	2·9	50–55
(*j*) Fructose + „ + „ (9·2; 5·2)	„	„	2·8–3·0	50

* These numbers indicate the P_H of that lot before and after autoclaving.

Table VI confirms the general results already outlined in connection with glucose (comparison of media *a-f*), together with evidence that the autoclaving of glucose at different P_H values has no effect on enzyme production. Comparison of media (*g–j*) shows that fructose also is improved from the point of view of enzyme production when autoclaved with K_3PO_4. There is also indication that fructose under comparable conditions gives somewhat stronger preparations than does glucose (compare *a–d* with *g–j* respectively).

When fructose is autoclaved with K_3PO_4 it behaves similarly to glucose in giving a brown yellow colour. The data of Table VI indicate that the amount of growth is greater, both for glucose and fructose, on the yellow type of medium. The same result is also suggested in Table V, but not shown in Tables III and IV.

No suggestion can be put forward in explanation of the beneficial effect obtained when phosphate is autoclaved with hexose sugars other than that some reaction product presumably catalyses growth and particularly enzyme production. The nearest approach to this result

is Hawker's (1943) statement that formation of perithecia by *Melanospora destruens* is stimulated by small quantities of certain phosphoric esters of glucose and fructose.

2. *Changes in Concentration, total or partial, and in Composition of Medium*

(i) *Effect of total concentration.*—The medium S, prepared with $MgSO_4$ autoclaved separately from the other constituents, was the standard one used, and concentrations over the range 2S–S/10 were tested for their enzymic activity after culture of isolate No. 2 for 5 days at 25° C. Table VII gives the results of a typical experiment. The medium marked "S with $MgSO_4$" was prepared by keeping phosphate separate from the rest during autoclaving. This comparison was usually included in these experiments, by way of confirming the special effect of the method of preparation, which has been described in the preceding section.

TABLE VII

Effect of Concentration of Medium on Enzymic Activity

Medium	Initial P_H	Final P_H	Dry wt. in units	R. T. mins.
2S	7·4–7·6	7·6–7·8	4–4·5	40–45
S	7·4	,,	3–3·4	30–35
S with $MgSO_4$	7·6–7·8	,,	2·9–3·3	85–90
S/2	7·4	7·4	1·9–2·2	50–60
S/5	7·2	7·4	1·5	90–100
S/10	7·4	,,	0·8–1·0	180

Table VII shows that whereas the amount of growth increases steadily from the weakest medium upwards, there is an optimum for enzymic activity in the neighbourhood of concentration S. Behaviour of P_H was not much affected over the range of concentration examined.

The summarised results of six other experiments carried out at various times on the same subject are collected in Table VIII. The data for P_H and for amount of growth are omitted as they followed the same lines as illustrated in Table VII.

The optimum of activity varied somewhat in its distinctness, but in all the experiments it was given by medium S.

Botrytis cinerea also gives active preparations of pectinase enzyme when grown on medium S and, therefore, for the sake of comparison, experiments were arranged to test the effect of the concentrations of medium S on its enzyme secretion. Media were seeded with spores

TABLE VIII

Effect of Concentration of Medium on Enzymic Activity

Experiments	2S	S	S/2	S/5	S/10
(1)	45	30	55	120	200
(2)	60	40	75	135	—
(3)	60	45	85	160	>240
(4)	45	35	60	105	190
(5)	40	30	50	90	—
(6)	90	35	65	180	>240

as described earlier and incubated at 25° C. for 5 days after which the filtrates were tested for their activity. Table IX gives the results of an experiment conducted with Pythium isolate No. 2 and Botrytis isolate No. 10 (p. 15).

TABLE IX

Comparative Study of Enzyme Secretion of Botrytis and Pythium

Medium	Initial P_H	BOTRYTIS			PYTHIUM		
		Final P_H	Dry wt. in units	R.T. mins.	Final P_H	Dry wt. in units	R.T. mins.
2S′	7·6–7·8	7·8	0·9	25–30	7·8	3·9	45
S′	,,	,,	0·4	15–20	,,	3·5	30
S″	8–8·2	,,	0·3	30	,,	3·2	85
S′/2	7·6–7·8	7·5	0·2	25	,,	2·3	55
S″/2	7·8–8	7·7	,,	40	—	—	—
S′/5	7·6–8	7·5	0·1	45	7·6	1·5	100
S′/10	,,	,,	<0·1	75	,,	0·8	>180

In the case of media marked ′, $MgSO_4$ was autoclaved separately; while with those marked ″, K_2PO_4 was autoclaved separately.

Table IX shows that the effect of concentration of S medium on enzyme secretion by Botrytis follows the same curve as in the case of Pythium, except that the peak of the curve is not so pronounced with the former. The experiment was repeated three times with Botrytis and in some of them the concentration for optimum activity was found

to range still more widely, there being, for example, in one experiment little difference over the range S/5–2S. Botrytis thus appears to be more tolerant than Pythium of concentration variations of the S medium. In addition to this effect Table IX shows that the favourable effect on enzyme secretion got by autoclaving phosphate and carbohydrate together is given also by Botrytis.

(ii) *Effect of proportions of various constituents*.—Experiments were set up to examine the effect of concentrations of carbohydrate in medium S on enzyme secretion. Media were prepared with nitrogen (asparagin and peptone) and salts (K_2PO_4 and $MgSO_4$) as in medium S and carbohydrate (glucose and starch together) concentration was varied over the range of 4S–S/5. Some difficulty was experienced, however, in preparing media with the higher concentrations of carbohydrate, *viz.*, 2·5S and 4S, as they tended to become sticky. Therefore, in later sets the highest concentration used was 2S. Table X gives the results of a relevant experiment, the cultural conditions being as usual.

TABLE X

Effect of Carbohydrate Concentration on Enzymic Activity

Media (starch and glucose concentration)	Initial P_H	Final P_H	Vol. mycel. cc.	R. T. mins.
4S	7·2–7·4	5·6–5·8	6·2	>240
4S'*	—	7·6–7·8	—	„
2·5S	7·4–7·6	6·4–6·6	4·6	200
2·5S'*	—	7·6–7·8	—	180
2S	7·4–7·6	6·4–6·6	4·9	145
2S'*	—	7·6–7·8	—	„
S	7·6–7·8	7·6–7·8	3·1	30
S/2	7·8–8·0	8·0–8·2	1·8	105
S/5	„	„	0·7	>240

* Each of these preparations is the same as the one immediately preceding, except that the P_H of extract was adjusted to approximately the optimum point before determination of R. T.

As the concentration of carbohydrate increases from S/5 upwards the amount of mycelium also steadily increases; but enzymic activity after similarly rising to a distinct peak in medium S falls off continuously in the higher concentrations. The drop in activity below S may be set down to poor growth while in the case of higher concentrations the P_H reached during growth, which is unfavourable to enzymic activity, might be partly responsible. Adjustment of P_H of these filtrates to optimum level (7·6–7·8) had no beneficial effect indicating

thereby that very little enzyme is secreted by the fungus under the experimental conditions or that it is being interfered with by some other factor. The results of three more experiments along the same lines have been given in Table XI. The data for growth and P_Hs have been omitted because they show the same general trend.

TABLE XI

Effect of Carbohydrate Concentration on Enzymic Activity

Experiment	4S	2·5S	2S	S	S/2	S/5
(1)	>240	175	120	30	130	>240
(2)	—	—	140	40	120	210
(3)	—	—	110	35	175	>240

In another set of experiments nitrogen (asparagin and peptone together) concentrations were varied upwards and downwards between 8S and S/5, carbohydrate and salts being kept constant at the level S. Exoenzyme filtrates were obtained from these media in the standard manner. The results are shown in Table XII.

TABLE XII

Effect of Nitrogen Concentration on Enzymic Activity

Media (asparagin and peptone concentration)	Initial P_H	Final P_H	Dry wt. in units	R. T. mins.
8S	6·3	7·7	3·5–4·0	50– 55
6S	6·9	7·1	3·6–3·7	45
4S	7·5	7·7	2·9–3·0	35
2S	7·8	7·8	2·7–2·9	65– 70
S	8·0	7·8	3·5–3·8	30– 35
S/2	8·2	7·8	2·6–2·8	70– 75
S/5	7·7	7·6	2·0–2·4	180–200

At concentrations of nitrogen lower than in S, enzymic activity follows the same course as in Table VII (p. 32) where total concentration was changed; above S however, enzymic activity does not fall off steadily as in Table VII but remains fairly constant as far as 8S. There is a suggestion of a second optimum at 4S and this is also shown in Table XIII where the summarised results of four experiments are given. With varied nitrogen the initial P_H in all these experiments is somewhat lower at higher concentrations (6S and 8S), but the final

P_H is much the same in all. Also there is no great variation in the amount of growth over the range of concentrations used, the determining factor no doubt being the concentration of carbohydrate which is the same throughout. Data relating to P_H and growth are therefore omitted in Table XIII.

TABLE XIII

Effect of Nitrogen Concentration on Enzymic Activity

Experiment	8S	6S	4S	2S	S	S/2	S/5
(1)	60	50	40	55	35	90	220
(2)	—	—	35	60	30	85	180
(3)	—	—	30	50	30	60	180
(4)	—	—	35	—	30	65	—

Though the same activity was shown by the enzyme of 4S and S in Table XII there is evidence that the enzyme derived from S is more concentrated than that from 4S. The following comparisons were made:—

4S	R. T. 35
Equal parts of 4S and water	„ 70–80
„ „ „ „ „ heat deactivated 4S	„ 80–90
S	„ 30–35
Equal parts of S and water	„ 50
„ „ „ „ „ heat deactivated S	„ 50–55
„ „ „ „ „ „ „ 4S	„ 55–60

It is clear that the S preparation is less reduced in activity by dilution than is the one from 4S. Thermostable retarding substances are not very prominent in any case, though there is a suggestion that deactivated 4S is more retarding than deactivated S, which again retards slightly more than water.

Table XIV gives the general effect on growth, P_H changes and enzymic activity when each constituent or group of these of the S medium are omitted in turn.

All the media are alkaline all along the period of growth, except the two (*h* and *j*) in which phosphate was omitted. These remain somewhat acid throughout. The amount of growth is cut down by leaving out any constituent, the most effective in this connection being starch, peptone and phosphate. The depressing effect of phosphate may be due, in part at least, to the unfavourable P_H reaction. As regards enzyme activity omission of any constituent reduces this, and out of all proportion to the corresponding reduction in growth (apart from media *d* and *g* where growth also is negligible). The lower

TABLE XIV

Effect of Omission of Constituent of S Medium upon Growth, P_H and Enzymic Activity

Media	Initial P_H	Final P_H	Dry wt. in units	R. T. min.
(*a*) S medium	7·8	8·0	3·0–3·5	40
(*b*) ,, without glucose	8·2	,,	2·5–2·7	80
(*c*) ,, ,, starch	7·8	,,	0·8–1·0	>300
(*d*) ,, ,, glucose and starch	8·4	8·2	0	>300
(*e*) ,, ,, asparagin	7·4	7·2	2·0–2·2	,,
(*f*) ,, ,, peptone	8·2	7·8	1·3–1·6	,,
(*g*) ,, ,, asparagin and peptone	7·2	7·4	0	,,
(*h*) ,, ,, K_3PO_4	5·5	5·8–6·2	1·5–1·6	,,
(*i*) ,, ,, $MgSO_4$	7·8	8·0	2·2–2·5	110
(*j*) ,, ,, K_3PO_4 and $MgSO_4$	5·5	5·8–6·2	1·2–1·7	>300

activity in absence of phosphate was not due to an unfavourable P_H, in the enzyme solution, because adjustment to P_H 8·0 did not give any improvement.

(iii) *Effect of substitutions.*—Various substitutions were made in the standard S medium, several types of carbohydrate and nitrogenous compounds being used. In all the media the salts were as in S. The media were made up as shown below.

The hydrolysed casein used was a purified product (Ashe Laboratories Ltd.), though it was stated to contain a trace of biotin; the sample which was unhydrolysed (Ashe Laboratories Ltd.) was probably less pure. The yeast was ordinary brewer's yeast in the form of dry pellets. In all the preparations the phosphate was autoclaved with the carbohydrate, but the characteristic brown colour was not produced with sucrose. The results are set out in Table XV.

The following media were made:—

(*a*)	Glucose,	0·5%;	starch,	2·5%;	asparagin,	0·45%;	peptone,	0·45%;			
(*b*)	„	3% ;	—	;	„	„ ;	„	„ ;			
(*c*)	Fructose,	„ ;	—	;	„	„ ;	„	„ ;			
(*d*)	Sucrose,	„ ;	—	;	„	„ ;	„	„ ;			
(*e*)	„	„ ;	—	;	„	„ ;	„	„ ;	yeast,	0·2%;	
(*f*)	„	„ ;	—	;	„	0·9% ;	—	;	„	„ ;	
(*g*)	„	„ ;	—	;	„	0·45%;	hydrolysed casein	0·45%;	—	;	
(*h*)	„	„ ;	—	;	„	„ ;	„	„ ;	yeast,	0·2%;	
(*i*)	„	„ ;	—	;	—	;	„	0·9% ;	„	„ ;	
(*j*)	„	„ ;	—	;	—	;	unhydrolysed casein	„ ;	„	„ .	

TABLE XV

Effect of Substitution on Enzymic Activity

		5 days incubation				10 days incubation		
Media	Colour	Initial P_H	Final P_H	Dry wt. in units	R. T. mins.	Final P_H	Dry wt. in units	R. T. mins.
(*a*)	Brown	7·8–8·0	7·7	3·5–3·7	30	7·8	3·0–3·2	40
(*b*)	,,	8·0–8·2	,,	4·0–4·7	40	,,	2·7–3·2	45
(*c*)	,,	,,	,,	4·5–5·1	35	,,	3·1	,,
(*d*)	Colourless	,,	5·7–6·8	2·0–2·2	80	7·5	3·5	65
(*e*)	,,	,,	7·5	3·5–4·0	65	7·7	3·5	45
(*f*)	,,	7·6–7·8	7·9	2·5–2·7	>180	7·9	1·6–2·1	>180
(*g*)	,,	8·0–8·2	5·7–6·5	1·1–1·5	>180	6·9	2·0–2·2	>180
(*h*)	,,	,,	7·3	3·0–3·5	70	8·1	2·9–3·1	45
(*i*)	,,	8·0–8·2	6·7–7·5	3·0–3·3	65	7·7	2·5–3·2	40
(*j*)	,,	7·6–7·8	7·9	2·5–2·7	180	7·9	1·6–2·1	180

The following conclusions may be drawn:—

(1) The replacement of starch by an equivalent amount of glucose (*a* and *b*) gives a weaker enzyme.

(2) Glucose alone (*b*) and fructose alone (*c*) are fairly comparable but sucrose (*d*) in the same concentration is distinctly inferior, though improved by addition of yeast (*e*).

(3) With sucrose as carbohydrate, and with asparagin and hydrolysed casein as source of nitrogen, growth and enzyme production are poor (*g*); both are improved by adding a small quantity of yeast (*h*); with asparagin and yeast, enzyme production is low (*f*), as also with unhydrolysed casein and yeast (*j*), while between asparagin and hydrolysed casein the latter seems to give better results (comparison of *f* and *i*).

There appears to be a correlation between the poor value of sucrose and the lack of development of the characteristic brown colour shown by glucose or fructose when autoclaved with K_3PO_4; there is also evidence that Pythium is benefited in growth and enzyme production by addition of a small quantity of yeast (comparsion of *h* and *g*); and in confirmation of this it was found in a preliminary experiment that the addition of 0·0025% of aneurin to medium (*g*) more than doubled the growth and gave a measurable enzymic activity (R. T. 90).

There is evidence, therefore, that Pythium tends towards the Nematospora type of organism which requires certain growth-promoting substances and which does not freely form these when grown in a simple synthetic solution (Buston and Pramanik, 1931).

Physico-Chemical Properties of Enzymes

In this work 4 types of enzymic preparations have, from time to time, been used, *viz.*,

(*a*) Pythium enzyme from medium S.

(*b*) *Pythium enzyme extracted from potato tuber tissue parasitised at 25° C.

(*c*) Botrytis enzyme from medium S.

(*d*) *Botrytis enzyme extracted from potato tuber tissue parasitised at 4° C.

Preparations (*a*), (*c*) and (*d*) were used in the crude form only, as the material of (*c*) and (*d*) was limited in amount and the purification of (*a*) resulted in considerable loss of activity. Preparation (*b*) was used both in the crude form and after 1–3 precipitations with acetone. This preparation was very active and maintained its original activity undiminished even after 3 precipitations.

(1) *Effect of* P_H *on Enzymic Activity*

The P_H range was set up by addition of N/10 NaOH or N/10 HCl and determinations made colorimetrically. Preparation (*b'*) was two times purified (*b*), (*c'*) was (*c*) diluted to $\frac{1}{4}$ concentration and (*c''*) was the crude preparation obtained by growing Botrytis on the following medium for 5 days at 25° C.:—

Ammonium tartrate	0·5%
Glucose	1·0%
KH_2PO_4	0·1%
$MgSO_4$	0·05%

On this medium the initial P_H is 5·6 and after 5 days' growth reaches to P_H 6·5, *i.e.*, it remains slightly acid throughout, in contrast to the alkaline behaviour on medium S (see p. 33). Table XVI gives the results.

All the preparations of Pythium enzyme (*a*, *b* and *b'*) have their optimal activity well on the alkaline side, at about P_H 8·0, and this applies also to the Botrytis preparations from medium S (*c* and *c'*) and from parasitised potato (*d*). It is noteworthy that the preparation (*c''*) from the acid medium has its optimum in the neighbourhood of P_H 5·5–6·0. This agrees with Fernando's (1937) claim that there is a certain adaptability to the conditions under which the enzyme is being secreted.

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* Some of the parasitised tubers from which preparation (*b*) was made and all of those from which (*d*) was obtained were handed over for this purpose by Mr. P. Isaac of this laboratory.

TABLE XVI

Effect of P_H *on Enzymic Activity*

Prep.	P_H 5·0	5·5	6·0	6·5	7·0	7·5	8·0	8·8	9·8
(*a*)	>180	>180	65	60	40	35	35	35	40
(*b*)	>180	135	60	45	30	30	30	30	30
(*b'*)	>180	130	60	50	30	30	30	30	30
(*c*)	>180	100	60	20	15	15	15	30	30
(*c'*)	—	>180	90	60	45	40	40	45	50
(*c''*)	45	35	35	45	50	60	90	>180	>180
(*d*)	—	50	—	25	—	25	25	35	>180

To a certain extent the effect of P_H on pectinase activity has also been determined by a viscosimetric method. For each determination 5 c.c. of 0·5% pectin* solution was adjusted to the approximate P_H value required by N/50 NaOH and kept at that P_H level by addition of 1 c.c. 0·4M Sörensen phosphate buffer and the volume in all cases made up to 9 c.c. by addition of distilled water. Then 1 c.c. of the enzyme was added to it, the mixture shaken and 5 c.c. of this was transferred to an Ostwald viscosimeter in water-bath maintained at 25° C. The viscosity changes were followed by observing flow times at regular intervals. Table XVII gives readings of the flow times, these being proportional to the viscosity of the pectin solution, of 4 preparations:—

(*a*) Pythium enzyme, crude, from S medium, tested at P_H 7·7.

(*b*) Pythium enzyme, once precipitated with acetone, from parasitised potato tuber, tested at P_H 7·7.

(*c*) Botrytis enzyme, crude, from S medium, tested at P_H 7·7.

(*d*) Botrytis enzyme, crude, from S medium, tested at P_H 5·6.

The figures in the table give the times, in seconds, for a standard volume of the liquid to pass through a fine tube under standard conditions. The higher the figure the greater the viscosity. The initial flow time reading of the pectin solution was 60·2 seconds.

The reaction times, as tested on standard discs (·5 mm. thickness) of potato, of Preparations (*a*) and (*b*) were 35 and 30 minutes respectively, and the latter further could be diluted with less falling off of activity than with (*a*). Nevertheless (*b*) is much less active in decreasing viscosity of pectin solution. The effect of P_H on acitvity of Botrytis

* Pectin used was Lemon Pectin, degree of methylation approx. 74·3% (by number), manufactured by General Foods Corp., Hoboken, N. J. This was washed 3–4 times with 60% alcohol containing 5% HCl, washed with 60% alcohol, finally with absolute alcohol and air dried.

TABLE XVII

Effect of Enzyme Preparations on Viscosity of Pectin Solution

Minutes after addition of enzyme	Prep. (*a*) Flow time in seconds	Prep. (*b*) Flow time in seconds	Prep. (*c*) Flow time in seconds	Prep. (*d*) Flow time in seconds
1	55·2	57·1	51·7	58·3
3	49·4	55·6	46·2	57·3
5	45·2	55·5	42·2	56·8
7·5	41·2	53·1	39·0	56·2
10	37·4	51·7	36·0	56·0
15	32·8	49·4	32·0	—
20	29·4	48·4	29·0	54·0
30	25·2	45·3	25·5	52·6

(The flow time reading for distilled water control at the beginning 60·2; after 15 minutes, 59·3).

enzyme is the same as tested by either method, *e.g.*, the optimum is at an alkaline point.

The effect of pectinase preparations in reducing viscosity of pectin solutions rests on a break-up of pectic aggregates. The relation of the latter to the cell-wall substances, which are attacked when tissue is macerated, is not clear from a biochemical standpoint. There may be different enzymes concerned in the process and the different behaviours of preparations (*a*) and (*b*) noted above could most readily be interpreted in this way.

(2) *Thermal Deactivation of Enzymes*

The method adopted was to heat the enzymic preparation in a water-bath to the temperature concerned, to maintain it at this point for 2 minutes and then to cool rapidly. The activity was then tested at 20° C. by the disc method.

The following preparations were examined:—

(*a*) Pythium enzyme from S medium.

(*b*) Pythium enzyme from parasitised potatoes.

(*c*) Pythium enzyme from parasitised potatoes purified with 2 acetone precipitations.

(*d*) Botrytis enzyme from S medium.

(*e*) Botrytis enzyme from parasitised potatoes.

The R.T.'s are given in Table XVIII.

TABLE XVIII

Determination of Heat Deactivation Point of Pythium and Botrytis Enzymes

Temp.	Prep. (*a*)	Prep. (*b*)	Prep. (*c*)	Prep. (*d*)	Prep. (*e*)
25° C.	35	30	30	25	20
30° C.	,,	,,	,,	,,	,,
35° C.	,,	,,	,,	,,	,,
38° C.	40	,,	,,	,,	,,
40° C.	45	40	45	,,	,,
45° C.	150	140	150	,,	,,
50° C.	>300	>300	>300	,,	,,
55° C.	,,	,,	,,	,,	,,
60° C.	,,	,,	,,	100	100
65° C.	—	—	—	>300	>300
70° C.	—	—	—	,,	,,

The deactivation point of Pythium enzyme is thus distinctly lower than that of Botrytis enzyme, *viz.*, 45–50° C. as against 60–65° C.

(3) *Effect of Crystalloids on Enzymic Activity*

Four substances were tested, *viz.*, two bivalent salts, $CaCl_2$ and $MgSO_4$; one monovalent salt, KNO_3; and a non-electrolyte, glucose. The enzymic preparations were those (*a–d*) given on page 41.

Addition of glucose or KNO_3, in the concentrations used, did not affect the P_H of the resulting solution. In the case of calcium or magnesium salts, however (especially between concentrations 0·01 M and 0·2 M), heavy precipitations were obtained with the natural extracts (*b* and *d*) together with the shift of P_H towards the acid side (5·8–6·2) while the preparations from the synthetic medium (*a* and *c*) gave little precipitation and had no P_H change; in the former case, therefore, the solutions were adjusted to the optimal P_H 7·6 by addition of N/10 NaOH before testing their activity. It may be added that there was very little difference in activity whether the P_H was adjusted or not.

Preparations (*a*) and (*b*) behaved more or less similarly as also did (*c*) and (*d*). The curves only for (*a*) and (*c*) are therefore given in Figs. 8 and 9 respectively.

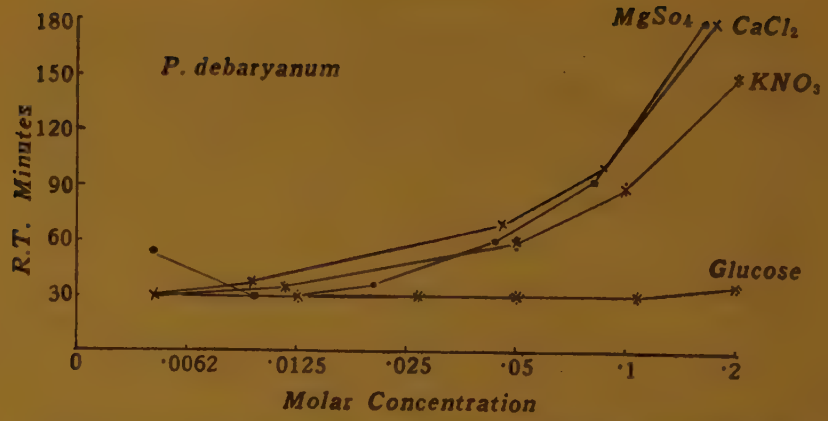

FIG. 8. Effect of crystalloids on enzyme of *Pythium debaryanum* (No. 2 obtained from S medium. Molar figures refer to the final concentrations after addition of equal volumes of the crystalloid solutions to the enzymic preparations.

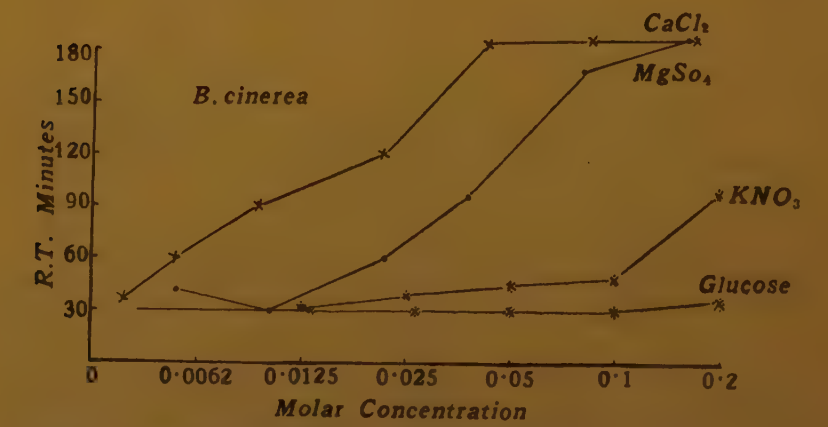

FIG. 9. Effect of crystalloids on enzyme of *Botrytis cinerea* (No. 10) obtained from S medium (Molar concentrations as in Fig. 8).

The enzymes of Pythium and Botrytis react in the same general way, *viz.*, they are more depressed by a given molar concentration of bivalent than of monovalent salts; glucose, in the concentrations tested, has no material effect on either.

For comparable concentrations of the various salts, Botrytis enzyme is more retarded than Pythium enzyme. These results fall into line with the observations of Brown (1915), Menon (1934), Ashour (1948), and Talboys (1950).

PARALLELISM BETWEEN ENZYME PRODUCTION AND PATHOGENICITY

Pathogenicity tests were carried out mainly with the three isolates of Pythium listed on page 15, *viz.*, *P. debaryanum* (No. 2), *P. intermedium* (No. 8) and *P.* species (No. 9). The hosts used were potato tubers (var. King Edward) and lettuce (var. Imperial) seeds and seedlings. For the sake of convenience the results will be described under two headings.

(1) *Pathogenicity Tests on Potato*

Whole potato tubers were inoculated and incubated (for 5–7 days) in the manner already described on page 16. Ten replicates were used for each isolate.

In preliminary work it was noted that *P. debaryanum* attacks potato tubers over the range of 10–30° C., *P. intermedium*, mostly between temperatures 10 and 25° C., and *P.* species is inactive at all these temperatures. Therefore, in most of the work the potatoes were incubated at 20° C., a favourable temperature for both the pathogenic species. Results of a typical experiment (incubation period 5 days) are given in Table XIX and summarised results of four other experiments are set in Table XX.

TABLE XIX

Comparative Pathogenicity (in gm. Rotted Tissue) of Three Pythium Species on Potato

Potatoes	Control	*P. debaryanum* (No. 2)	*P. intermedium* (No. 8)	*P.* species (No. 9)
1	0	35	0	0
2	0	88	0	0
3	0	70	15	0
4	0	17	0	0
5	0	87	17	0
6	0	70	39	0
7	0	101	0	0
8	0	72	0	0
9	0	89	12	0
10	0	23	0	0
Average	0	65·2	8·3	0

P. debaryanum proved to be virulent pathogen producing soft rot of potatoes very rapidly. *P. intermedium* was comparatively weaker,

though it was definitely capable of attacking potato tissue, as it developed soft rot in every set of experiment arranged during these tests (see Table XX). *P.* species never produced rot under the experimental conditions.

TABLE XX

Comparative Pathogenicity (in gm. Rotted Tissue) of Three Pythium Species on Potato

Expt.	Control	*P. debaryanum* (No. 2)	*P. intermedium* (No. 8)	*P.* species (No. 9)
(1)	0	61	11	0
(2)	0	53	9	0
(3)	0	65	6	0
(4)	0	69	13	0

Similar experiments were carried out on a more limited scale with potato plugs (1·8 × 10 cm.) cut with a large cork borer (p. 17) in place of whole potatoes. They were incubated for 2 days. The comparative behaviour of the isolates was the same as just described. Table XXI gives the results of two such experiments.

TABLE XXI

Comparative Pathogenicity (in gm. Rotted Tissue) of Three Pythium Species on Potato

Expt.	Control	*P. debaryanum* (No. 2)	*P. intermedium* (No 8)	*P.* species (No. 9)
(1)	0	9·5	4	0
(2)	0	10	1	0

Side by side with the foregoing, experiments were done with autoclaved potatoes. It was interesting to find that none of these developed the typical watery soft-rot either with *P. debaryanum* or *P. intermedium*. However, unlike the surface sterilised potatoes (except for occasionally a little growth at the inoculation point), the fungi grew all over the potato externally in large cottony tufts which increased with the incubation period. When cut the potato showed the mycelium within but they did not turn dark brown, soft and watery nor on pressing did they yield the characteristic deep brown extract which was easily obtained from surface sterilised parasitised potatoes. When these autoclaved parasitised potatoes or plugs were squashed in a little water and the debris removed by centrifusing, the liquid usually gave a R.T. of about 200–240 minutes, or even longer, as against 25–30 minutes

given by the extract of surface sterilised parasitised potatoes. It may be added that the latter maintained more or less the same R.T. even up to $\frac{1}{4}$ of its dilution, thus showing it to be of high enzymic concentration. These results are not in full agreement with those of Menon (1934) and Fernando (1937), who found fairly similar activities in extracts prepared in these two ways.

On several occasions other isolates of Pythium (p. 15) were included in these pathogenicity tests. In general, isolates Nos. 1, 3, 6 and 7 agreed with *P. debaryanum* (No. 2) and No. 5 with *P. intermedium* (No. 8). No. 4 which is also of intermedium type occasionally gave no attack at all in the 10 replicates, thus showing itself to be a weaker pathogen than Nos. 5 and 8.

(2) *Pathogenicity Tests on Lettuce*

These tests were carried out with sand- or soil-cornmeal cultures (p. 18) and they were seeded or planted (2–3 weeks old seedlings) with lettuce. Three replicates were made in each case. They were maintained at greenhouse temperature (17–20° C.) for 8–10 days and then examined for the damping off. The results of three experiments with soil- and sand-cornmeal cultures are given in Table XXII.

TABLE XXII

Pre- and Post-Emergence Damping off of Lettuce by Pythium Isolates

(per 25 seed sown or 15 plants planted)

	P. debaryanum (No. 2)	*P. intermedium* (No. 8)	*P.* species (No. 9)	Control
SEEDS				
Soil-cornmeal	1–3 Em. Root tips brown	15–18 Em. 4–5 damped, 5–6 root tips brown	21–23 Em. Occasional damped off, or root tip brown	21–23 Em. None
Sand-cornmeal	3–5 Em. Root tips brown	15–20 Em. 5–7 damped, 4–5 root tips brown	20–23 Em. Occasional damped off, or root tip brown	20–23 Em. None
PLANTS				
Soil-cornmeal	7–9 D. O. 2–4 with root tips brown	5–8 D. O. 3–5 with root tips brown	Occasional D. O. or root tip brown	None
Sand-cornmeal	6–8 D. O. 2–4 with root tips brown	4–8 D. O. 2–5 with root tips brown	,,	,,

Em = Emergence; D.O. = Damping off.

Similar sowings or plantings made on plain agar cultures of these Pythium isolates behaved in the same way, but gave results even more

quickly. Those of 25 seed sown or of 15 plants planted, all died within 2–3 days on a plate of *P. debaryanum*; on a plate of *P. intermedium* only 4–6 seed or 6–9 plants had died in 5–7 days, the remaining ones showing brown root tips; on *P.* species only an occasional seed or plant was killed. Seed placed on *P. debaryanum* culture only sometimes showed initial germination, but they invariably became rotted.

The general conclusion, therefore, is that under all the conditions tested *P. debaryanum* was highly pathogenic to lettuce seed or seedlings, *P. intermedium* much less so and *P.* species not at all, the results thus falling into line with those for attack on potatoes.

(3) *Enzyme Secretion by Pythium Isolates*

The enzymatic behaviour of *P. debaryanum* (No. 2), *P. intermedium* (No. 8) and *P.* species (No. 9) on various concentrations of the S medium is given in Table XXIII. The P_H range in the various media was from 7·6–7·8 and these details are, therefore, omitted. The cultures were tested for their enzymic activity after 5, 10 and 15 days incubation. The table shows R.T.'s after 5 days growth, at 20° C.

TABLE XXIII

Enzyme Secretion by Pythium Isolates

Concentration	*P. debaryanum* (No. 2)		*P. intermedium* (No. 8)		*P.* species (No. 9)	
	Dry wt. in units	R.T. mins.	Dry wt. in units	R.T. mins.	Dry wt. in units	R.T. mins.
2S	3·5–4·0	40	2·7	>180	2·0–2·2	>180
S	2·9–3·2	30	1·4–2·0	>180	1·6–2·0	,,
S/2	2·0–2·2	55	0·9–1·3	150	1·0–1·2	,,
S/5	1·3–1·7	95	0·8–1·1	60	0·6–0·9	,,
S/10	0·6–1·0	>180	0·8–1·0	95	—	—

The growth vigour of *Pythium debaryanum* is somewhat greater than that of the other two species, but the main difference is in the greater enzymic activity of *P. debaryanum*. *P. intermedium* gave a fairly active preparation on dilute media (S/5), which became still more active after 10 days; after that it fell away. *P.* species gave no measurable activity at any concentration of the medium, and the same was true after 10 and 15 days. The broad result, therefore, is that greatest enzymic activity is shown by cultures of *P. debaryanum*, and least by those of *P.* species.

Experiments on the same scale were set up on various concentrations (50%, 20%, 10%) of potato extract and the three lettuce decoctions containing 5%, 2·5%, 1·25% of leaf substance. None of the

potato extracts gave appreciable enzymic activity with any of the three fungi, except for *P. debaryanum* on the 10% extract, the activity recorded being quite weak (R.T. 180). On the lettuce decoctions, only *P. debaryanum* gave active preparations; *viz.*, on 5%, R.T. 70; on 2·5%, R.T. 125. On all the remainder no activity was demonstrable. It should be added that on the lettuce decoctions all the isolates grew rather poorly.

The results with potato and lettuce decoctions are, therefore, so far as they go, in full agreement with those on medium S. There is, therefore, a distinct parallelism between the capacity to parasitise potato and lettuce tissues and to produce pectinase enzyme.

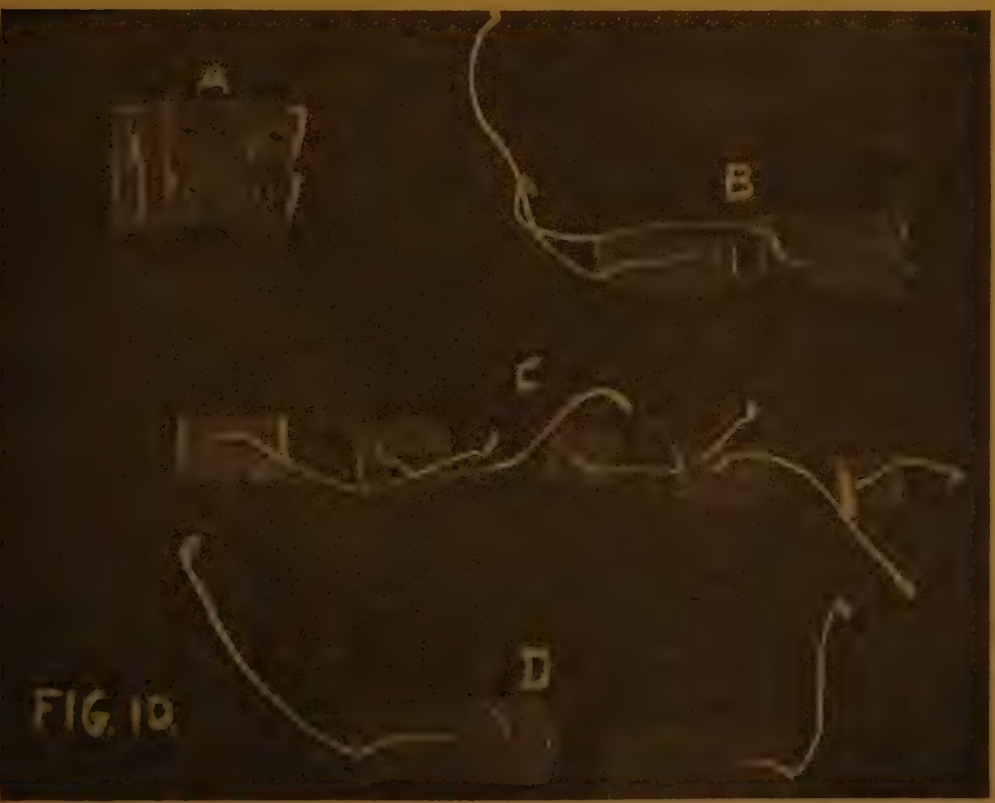

FIG. 10. Effect of mixed culture of two species of Pythium on their pathogenicity on lettuce (after eight days' incubation at 25° C.)

A. Portion of *P. debaryanum* culture with parasitised seeds.

B. Portion of P. species culture with unaffected germinated seedlings.

C. Portion of dual culture (*P. debaryanum* P. species) with almost normal germinated seedling; some of them show root injury.

D. Portion of plain agar with normal, healthy germinated seedlings (control).

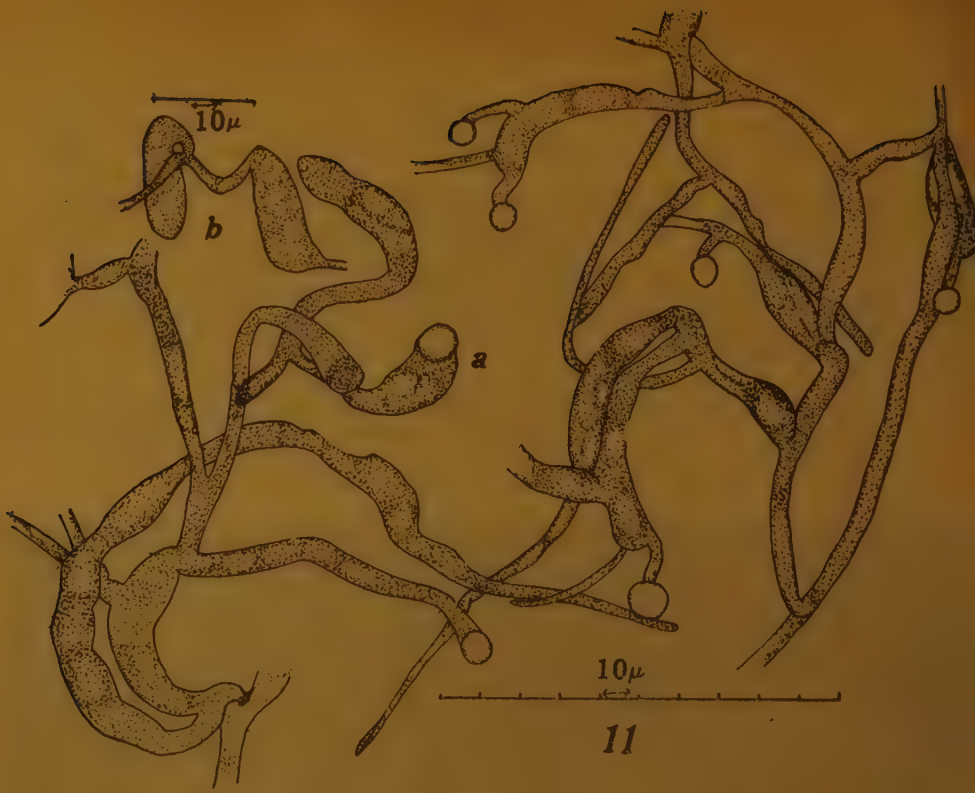

FIG. 11. Appressoria of Pythium. Except the portion marked " b " the whole figur shows appressoria of *P. debaryanum*. Note their fascicled, elaborate nature as compare to the ones produced by *P. intermedium* (*b. a* shows the portion of hypha where the fungu has made an intimate, discoid contact with glass.

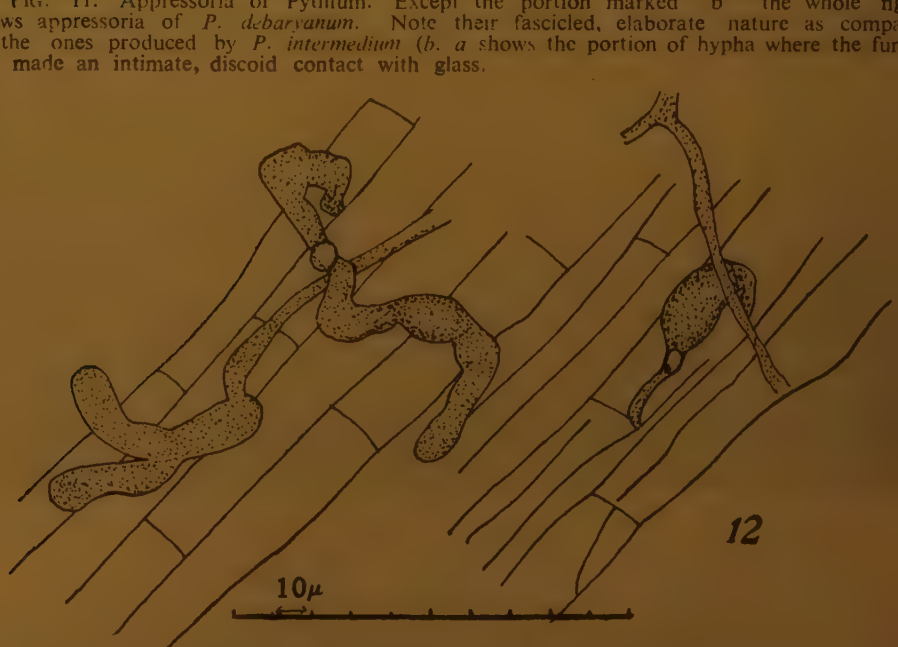

FIG. 12. Appressoria of *P. debaryanum* produced on the stem of lettuce.

In the cultural comparison of Pythium isolates it was pointed out that there was marked antagonism between P. species and any isolate of *P. debaryanum* or *P. intermedium*, this taking the form that P. species readily grew through cultures of the other two types, the latter however, did not grow through a culture of P. species. A similar effect is shown in respect of pathogenicity. An experiment of this kind, which is illustrated in Fig. 10, was carried out by inoculating plates of plain agar in four ways:

(1) With *P. debaryanum* at the centre and alone,
(2) With *P.* species at the centre and alone,
(3) With *P. debaryanum* and *P.* species mixed, at the centre,
(4) With *P. debaryanum* and *P.* species at diametrically opposite points.

The cultures were allowed to grow 6 days by which time they had filled the plates, then they were sown with surface sterilised lettuce

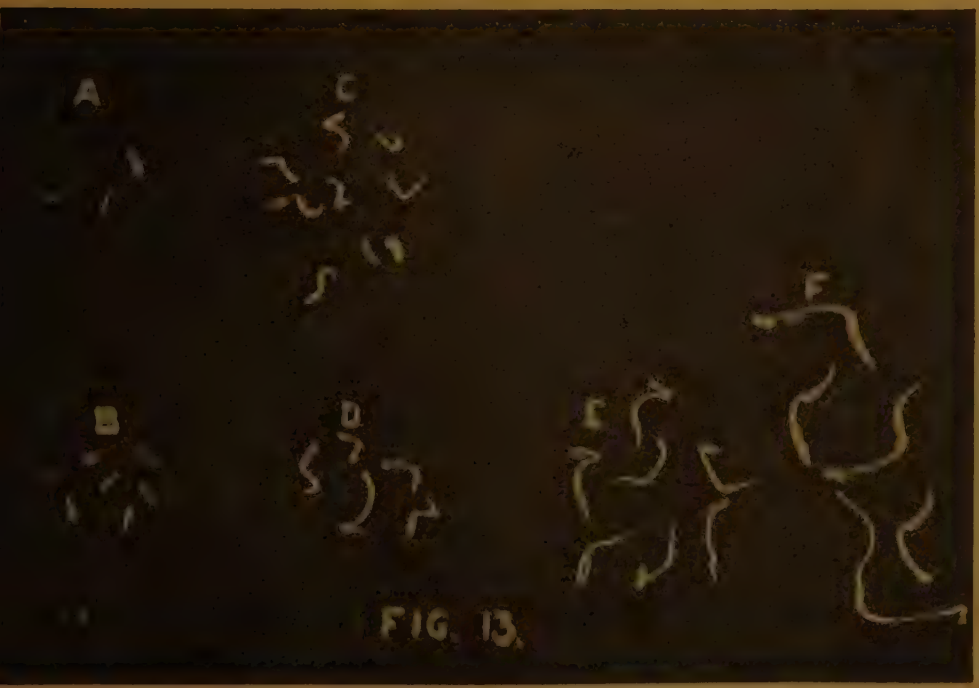

FIG. 13. Effect of active and heat deactivated enzyme preparations on germination of lettuce seed, after four days' treatment.
A. Seeds in enzyme extract from parasitised potato.
B. Seeds in deactivated Extract from parasitised potato.
C. Seeds in enzyme preparation from S medium.
D. Seeds in deactivated preparation from S medium.
E. Seeds in fresh S medium.
F. Seeds in water.

seed or planted with seedlings. After 8 days incubation at 20° C. the condition illustrated in Fig. 10 was shown.

On *P. debaryanum* alone all seeds and seedlings were killed (Fig. 10 A), on *P.* species alone seeds and seedlings grew (Fig. 10 B) much as on the plain agar controls (Fig. 10 D). On the mixed cultures the growth was not markedly different (Fig. 10 C) from that on the plain agar controls or on the culture of *P.* species, some of the seedlings being normal but others with a certain amount of damage to the root tip. The presence of P. species in the mixed culture has clearly much reduced the parasitic activity of *P. debaryanum.*

Connected with pathogenicity is the capacity to produce appressoria. Both *P. debaryanum* and *P. intermedium* produce these when grown

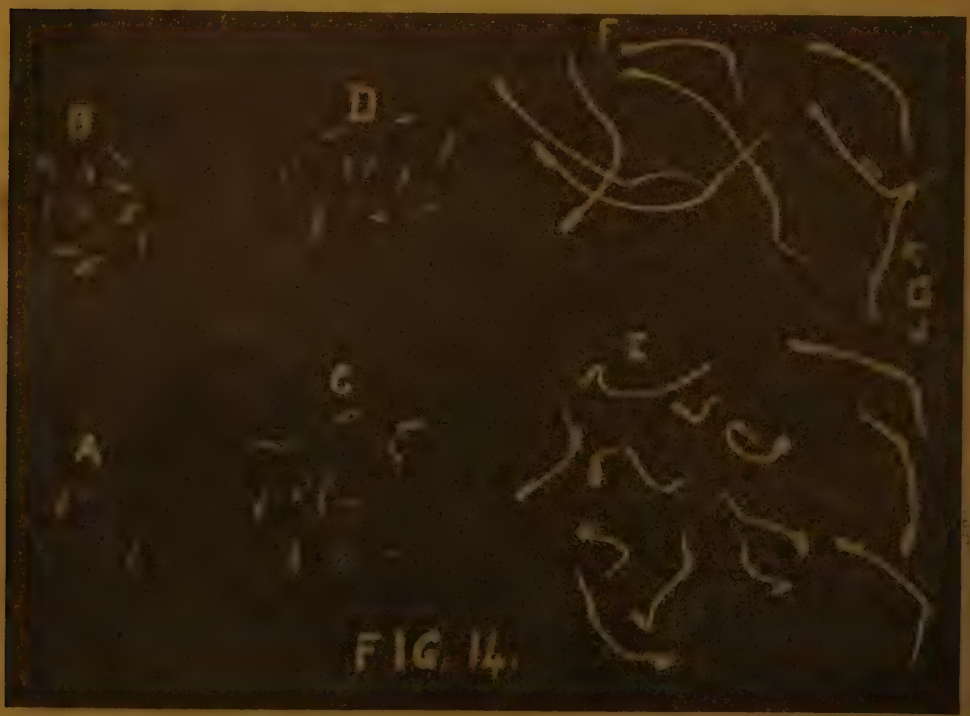

FIG. 14. Effect of active and heat deactivated enzyme preparations on germination of lettuce seed, after four days' treatment.

A. Seed in enzyme extract from parasitised potato.
B. Seed in deactivated extract from parasitised potato.
C. Seed in once acetone purified enzyme extract from parasitised potato.
D. Seed in deactivated once acetone purified enzyme extract from parasitised potato.
E. Seed in potato juice.
F. Seed in once acetone purified potato juice.
G. Seed in water.

in plain agar and when their hyphæ come in contact with glass; and *P. debaryanum* has been seen to produce them on the surface of lettuce stems (Figs. 11 and 12). P. species has not been found to develop them in any circumstances.

Evidence was obtained that a toxic substance is present in culture media of *P. debaryanum* in addition to pectinase enzyme. The poisoning effect is thus shown in boiled solutions, the toxin being thermostable. Figs. 13 and 14 illustrate the results.

Fig. 13 A shows lettuce seed which had been placed 4 days in extract from potato tubers parasitised by *P. debaryanum*; B shows seed which had been placed for the same time in autoclaved extract. In both cases the seed had been killed, there being no germination even after washing and plating. These seeds also developed moulds on standing. (C) and (D) are seeds which had been given similar treatment in the extract from culture on S medium. The effect here is intermediate. (E) and (F) are the controls on fresh S medium and on water.

In Fig. 14, A and B are as C and D are for the same enzymic preparation purified by one precipitation with acetone; E gives the effect of potato juice only; F is potato juice purified by one precipitation with acetone; G is the water control.

These results indicate the presence in Pythium extract of a thermostable toxin which is not removed by one precipitation with acetone.

Summary

1. A cultural comparison has been made of 27 isolates of Pythium. These have been shown to fall into four species. An interesting case of antagonism between one species and the others is described. A selection of these isolates together with five named Pythium cultures and one of *Botrytis cinerea* have been further studied from the point of view of their capacity to produce pectinase enzyme in a variety of media.

2. It has been found that the method of preparing a particular synthetic medium for the culture of these fungi for enzyme secretion has a marked effect on enzyme production. To obtain an active preparation of exoenzyme alkaline or neutral phosphate is required to be autoclaved with the carbohydrate of the medium.

3. The order of preference of Pythium for carbohydrate is fructose, glucose, starch and sucrose in diminishing series.

4. Dilution or concentration (or omission) of carbohydrate or nitrogen constituents of the standard medium reduces the enzyme secretion of Pythium (less so in the case of concentration of nitrogen), optimum being at the level present in the standard medium. Thus Pythium is selective in its nutritive requirements.

5. Enzyme extracts are also obtained from living potato tubers parasitised by Pythium (at 25° C.), and by Botrytis (at 4° C.) and are found to be extremely active inasmuch as they maintained the same

tissue-macerating activity even when diluted to one-fourth of their original concentration.

6. The optimum P_H for Pythium enzyme lies in the neighbourhood of P_H 8·0; that of *Botrytis cinerea* depends on the medium from which it was prepared, being at an acid point (P_H 5·6), when the culture medium was such that it remained acid throughout, but at P_H 8·0 when the fungus was cultured on an alkaline medium.

7. The thermal deactivation point of Pythium enzyme is unusually low, *viz.*, at 45–50° C. (after 2 minutes' heating), as compared with 60–65° C. for Botrytis enzyme under the same conditions.

8. Enzymic preparations of Pythium and Botrytis are insensitive to the presence of glucose, but are retarded by KNO_3 and still more by bivalent salts such as $CaCl_2$ and $MgSO_4$; Pythium enzyme being less sensitive than Botrytis enzyme.

9. A parallelism has been observed between the capacity of the Pythium species to produce pectinase enzyme and their ability to parasitise potato and lettuce tissues. The most active species in both respects were *P. debaryanum* and *P. ultimum*; *P. intermedium* was less so, and a species with spiny oogonia (*P. mastophorum* or *P. polymastum*?) was quite inactive.

10. In addition to the tissue-macerating enzyme pectinase, cultural extracts of Pythium contain a thermostable toxin having a lethal effect on the plant tissue.

Acknowledgements

The present investigation was carried out at the Imperial College, London. The writer wishes to express his indebtedness to Prof. W. Brown, D.Sc., F.R.S., who suggested the subject of this research and offered valuable criticism during its progress. Thanks are due to Mr. Tooley for his help in taking the photographs.

Literature Cited

Abdel-Salam, M. A. 1933. Damping Off and Other Allied Diseases of Lettuce. J. Pomol. and Horti. Sci. 11: 259–75.

Ashour, W. E. 1948. The Effect of Cultural Conditions on the Production of Pectinase by *Botrytis cinerea* and *Pythium debaryanum*. Thesis in Botany Library, R.C.S.

Brandenburg, E. 1948. Über ein Pilzliches Toxin in der Gattung Pythium und seine Wirkung auf die Wirtspflanze. Zeitschr. Pflanzenkr. 55 (5/6): 129–38.

———. 1950. Über die Bildung von Toxinen in der Gattung Pythium und ihre Wirkung auf die Pflanzen. Nachr. Bl. dtsch. Pflsch. Dienst. (Braunschw), 2 (5): 69–70.

Brown, W. 1915. Studies in the Physiology of Parasitism. I. The Action of *Botrytis cinerea*. Ann. Bot. 29: 313–48.

Buston, H. and Pramanik, B. N. 1931. The Accessory Factors Necessary for Growth of *Nematospora gossypii*. I Bio-Che. Jour. 25: 1656–70.

Chona, B. L. 1932. Studies in the Physiology of Parasitism. XIII. An Analysis of the Factors underlying Specialization of Parasitism, with Special Reference to Certain Fungi Parasitic on Apple and Potato. Ann. Bot. 46: 1033–50.

DRECHSLER, C. 1943. Antagonism and Parasitism among Some Oomycetes Associated with Root Rot. J. Wash. Acad. Sci. 33: 21–28.

FERNANDO, M. 1937. Studies in the Physiology of Parasitism. XV. Effect of the Nutrient Medium upon the Secretion and Properties of Pectinase. Ann. Bot. (N. S.). 1: 727–45.

HAWKER, L. E. 1948. Stimulation of Formation of Perithecia of *Melanospora desdruens* by Small Quantities of Certain Phospheric Esters of Glucose and Fructose. Ann. Bot. (N. S.). 12: 77-79.

HAWKINS, L. A. 1916. The Disease of Potatoes known as "Leak". Z. Agr. Res. 6: 627–40.

——— AND HARVEY, R. B. 1919. Physiological Study of the Parasitism of *Pythium debaryanum* Hesse on the Potato Tuber. J. Agr. Res. 18: 275–97.

JACKS, H. 1951. The Efficiency of Chemical Treatments of Vegetable Seeds against Seed-borne and Soil-borne Organisms. Ann. Appl. Biology. 38: 135–68.

MATTHEWS, V. D. 1931. Studies on the Genus Pythium.

MENON, K. V. P. 1934. Studies in the Physiology of Parasitism. XIV. Comparison of Enzymic Extracts Obtained from Various Parasitic Fungi. Ann. Bot. 48: 187–200.

MIDDLETON, J. T. 1944. The Taxonomy, Host Range and Geographic Distribution of the Genus Pythium. Memoirs of the Torrey Botanical Club. 20: 1–171.

SIDKY, S. T. 1947. Pre-Emergence Damping Off of Lettuce Seedlings. Effect of Environmental Conditions on the Persistance and Activity of the Parasite, with Experiments on the Value of Copper Fungicides as Seed Protectants. Thesis in Botany Library, R. C. S.

SMIETON, M. J. AND BROWN, W. 1940. Botrytis Disease of Lettuce: Its Relation to Damping off and Mildew, and Its Control by Pentachloro-nitrobenzene Dust. Ann. Appl. Biology. 27: 489–501.

TALBOYS, P. W. 1950. A Comparative Study of the Enzymic Mechanism of Certain Plant Pathogens. Thesis in Botany Library, R.C.S.

TOLBA, N. K. 1949. Physiological Analysis of Pathogenicity of Certain Plant Parasitic Bacteria. Thesis in Botany Library, R.C.S.

A CONTRIBUTION TO THE EMBRYOLOGY OF *TRIUMFETTA RHOMBOIDEA* JACQ. AND *CORCHORUS ACUTANGULUS* L.

By C. Venkata Rao and K. V. Sambasiva Rao

Department of Botany, Andhra University, Waltair

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The previous embryological work on Tiliaceæ is very meagre. The observations on the structure of the ovule and development of the male and female gametophytes in a few genera like *Tilia*, *Sparmannia* and *Entelæa* by Warming (1878), Strasburger (1884), Juel (1915), Stenar (1925), Sommer (1929) and Wallisch (1930), have been summarised by Schnarf (1931). Banerji (1933 *a* and *b*) studied the cytology, development of the male and female gametophytes and fertilisation in the two cultivated species of jute, namely, *Corchorus olitorius* and *C. capsularis*. He found, as in other genera of the family previously investigated, that the anther tapetum is of the secretory type and cytokinesis of the microsporocytes is brought about by furrowing. The pollen grains are 3-porate and are shed at the 2-nucleate stage. The development of the embryo-sac follows the normal-type. The antipodals are ephemeral and the polar nuclei do not fuse till the time of fertilisation. Stenar (1925) who studied the development of the embryo in *Tilia platyphyllos*, reported that the cell divisions do not follow any regular sequence (quoted from Schnarf, 1931), but Souèges (1941) who re-investigated it, found that as in other Malvales, it conforms to the *Urtica* variation of the Asterad Type. No doubt there are a few deviations in the segmentation of the basal cell of the 2-celled proembryo, but as Johansen (1950) remarks, " these are not sufficiently important to require the erection of a different variation". The few stages of embryo development sketched by Stenar (1925) in *Entelæa palmata* and Banerji (1933) in *Corchorus olitorius* as well show that the embryo development in them follows the same type as in *Tilia platyphyllos*.

The present paper deals with the devleopment of the male and female gametophytes in *Corchorus acutangulus* L. and *Triumfetta rhomboidea* Jacq. and the embryogeny in the latter species. The material of both the species was collected from plants growing as weeds in the Andhra University grounds. It was fixed in formalin-acetic-alcohol. Heidenhain's iron-alum-hæmatoxylin and a combination of safranin and fast green were used as stains.

Organogeny

The sequence in the development of the floral organs in *Triumfetta rhomboidea* is bract, calyx, androœcium, gynœcium and lastly corolla (Figs. 1–6). The sepals grow rapidly, become boat-shaped and encircle and protect the developing stamens. The petals make their appearance only when the microspore mother cells in the stamens have reached

the late prophase I. They show very tardy growth, reaching the height of the filaments when the microspores are 2-nucleate. Their growth speeds up just before the opening of the flower buds. Banerji (1933 *a*) reported that in the *Corchorus* species investigated by him, the floral organs arise in strict acropetal sequence but the growth of the petals is arrested till the microspore formation is completed. As Rao (1950) pointed out in *Waltheria indica* L., the belated appearance of the petals and their tardy growth may be indicative of the tendency for their suppression which culminates in the apetalous genera of the Heteropetalæ section of the family.

Microsporogenesis and Male Gametophyte

A row of 8–10 hypodermal archesporial cells differentiates in each of the 4 lobes of the young anther. These cells, by a periclinal division, form the primary parietal cells to the outside and the primary sporogenous cells to the inside (Fig. 7). The primary parietal cells undergo a periclinal division and become 2-layered. By a division in the inner layer of cells in *Triumfetta rhomboidea* and the outer layer in *Corchorus acutangulus*, the anther wall ultimately becomes 4-layered including the epidermis (Figs. 9 and 11). The subepidermal layer develops into the fibrous endothecium (Fig. 10) and the innermost into the tapetum which is of the secretory type. Its cells are commonly 2-nucleate, 3- or 4-nucleate cells being found rarely (Fig. 12). The epidermal cells of the fresh anthers of *Triumfetta rhomboidea* show rosette-shaped crystals. These are not seen in sections of fixed materials, but the cells show droplets of some deep staining material as in several genera of Sterculiaceæ (Fig. 10).

The primary sporogenous cells undergo repeated mitotic divisions to form several microspore mother cells. Just before the meiotic divisions, there are 3–6 cells in t.s. and 10–12 cells in l.s. of an anther loculus. The nucleolus of the microsporocyte has a deeply chromatic peripheral zone and a vacuole-like central region, inside which is found a crystalline body (Fig. 13). Rao (1941) reported a similar structure for the nucleoli of the root tip cells and microsporocytes of *Hibiscus trionum* L. The meiotic divisions proceed normally with a distinct interphase (Figs. 14–20). The number of bivalents counted in prophase I in the microsporocytes of *Triumfetta rhomboidea* is 24; the same number of univalents is counted from the metaphase plates of meiosis II. So the chromosome number in this species is $2n = 48$ and $n = 24$. Though usually all the cells of a loculus are at the same stage of division, sometimes a marked disparity is seen as shown in Fig. 11. The wall of the microsporocyte gets disorganised during prophase I and the protoplast gets invested by a special wall of callose which persists till the microspores are formed. The spores are arranged in a tetrahedral fashion (Fig. 18), bilateral tetrads being rare (Fig. 19). Cytokinesis is brought about by the development of centripetal furrows (Fig. 20).

The young microspore when liberated from the special wall is small and lenticular. It shows rapid growth and soon becomes

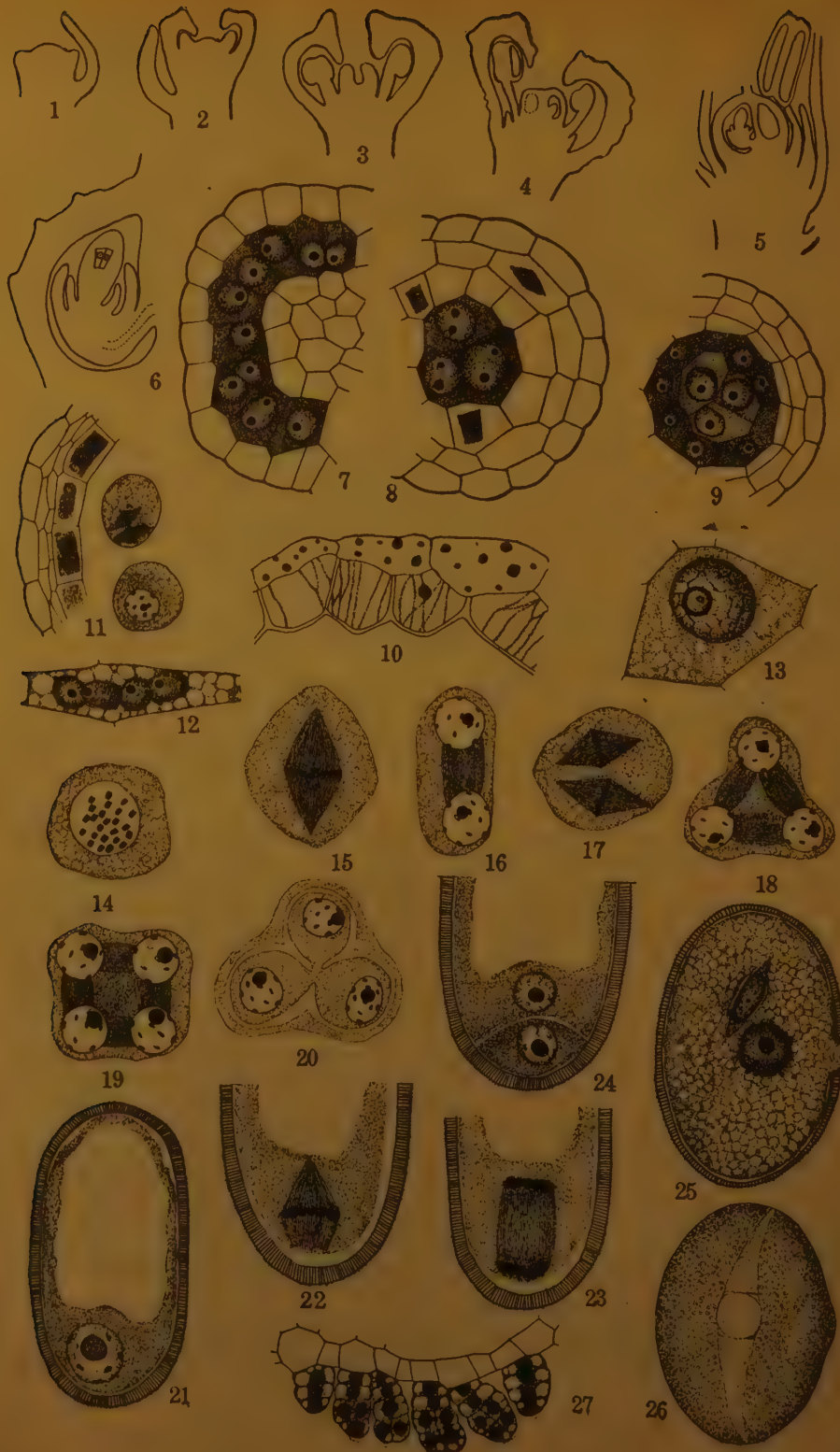
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Fig. 9. *Corchorus acutangulus*. Figs. 1–8 and 10–27. *Triumfetta rhomboidea*.— Figs. 1–6. Stages in the organogeny of the flower. Fig. 7. L. S. anther lobe showing archesporium and formation of primary parietal cells. Fig. 8. T. S. same showing formation of parietal layers. Fig. 9. T. S. anther lobe of *Corchorus acutangulus* showing sporogenous cells and tapetum. Fig. 10. Fibrous endothecium. Fig. 11. T. S. of anther showing wall layers, tapetum and two microsporocytes in different stages of division. Fig. 12. A multinucleate tapetal cell. Figs. 13–20. Stages in meiotic divisions. Fig. 21. Microspore with nucleus in prophase. Figs. 22–24. Stages in division of microspore nucleus; note the asymmetrical spindle. Figs. 25 and 26. Sectional and surface views of mature pollen grains. Fig. 27. Portion of a 'staminodal nectary' at the base of a petal. Figs. 1–3, × 45; Figs. 4 and 5, × 30; Fig. 6, × 90; Figs. 7–12, × 700; Figs. 13–26, × 1090; Fig. 27, × 375.

ellipsoidal. The nucleus comes to lie at one end of the pollen grain. The cytoplasm on the generative pole of the nucleus is scanty but dense, while that on the vegetative pole shows a large vacuole. Division of the microspore nucleus was followed closely in *Triumfetta rhomboidea*. The spindle in both metaphase and anaphase stages was markedly asymmetrical; it is top-shaped with pointed vegetative and rounded generative poles (Fig. 22). Hagerup (1938), on the other hand, found that in *Orchis* the anaphase spindles are normal but those of metaphase and telophase are asymmetrical. The asymmetry is due to the alignment of the vegetative chromosomes in a hemispherical manner. Brumfield (1941) who made a special study of the asymmetrical spindles in *Allium*, *Pancratium* and *Tradescantia*, associates them with the flattened shape of the prophasic nucleus. But this cannot be the cause in *Triumfetta*, because the nucleus at prophase was found to be normal and spherical (Fig. 21). On the other hand, the shape of the spindle seems to be due to the scanty cytoplasm on the generative pole and its rounded outline as Caldwell (1899) found in *Lemna*.

The generative cell, which is lenticular when first formed (Fig. 24), rounds up after the ephemeral partition disappears. It migrates into the vegetative cytoplasm and its nucleus assumes an ellipsoidal shape by the time it moves to the equatorial region of the pollen grain. It is invested on the sides by the sheath of hyaline generative cytoplasm. At the two ends are found two cap-like zones of denser cytoplasm in which fibre-like striations can be seen. These, however, are not the spindle fibres, since they are seen even when the nucleus is in the metabolic stage. By the time the pollen grains are shed, the nucleus attains the prometaphase stage in which the outline of the chromosomes becomes distinct (Fig. 25). The division of the generative nucleus is completed inside the pollen tube while it is making its way through the stylar tissues as in most Malvales. The cytoplasm of the pollen grain is packed with starch grains.

Mature pollen grains are ellipsoidal in both species. The exine is reticulately thickened; the thickenings appear as radial bars in sections. There are 3 germ pores, one each in the middle of a fusiform germinal furrow. The intine protrudes slightly through the germ pores (Fig. 25).


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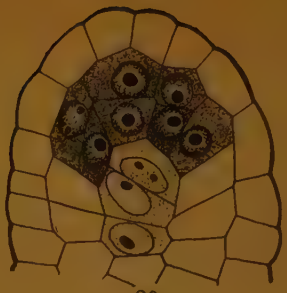
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FIGS. 28–40. *Triumfetta rhomboidea.*—Figs. 28–32. L. S. young ovules showing archesporium and formation of parietal tissue. Fig. 33. Ovule showing integuments and 2 functional megaspore mother cells. Fig. 34. Formation of linear tetrad. Figs. 35–38. Early stages in development of the embryo sac and degenerating tetrads. Fig. 39. Fertilisable embryo sac. Fig. 40. L. S. mature ovule. Figs. 28–39, × 425; Fig. 40, × 200.

OVARY AND OVULE

The ovary in *Triumfetta rhomboidea* is 2-carpellary and syncarpous. It is 4-locular at base and 2-locular at the top, since the midrib of each carpel forms an incomplete septum. Glandular outgrowths develop on the ovary wall when the ovules are in the megaspore-mother cell stage (Fig. 6); these become hook-like in the fruit and assist in its dispersal. Ovules are axile and basal in origin. As they grow and become anatropous, the axial region of the ovary below their attachment elongates so that the mature ovules become pendulous and show ventral raphe. The ovary terminates in a filiform solid style.

The ovary in *Corchorus acutangulus* L. is elongated, 3-carpellary, 3-locular and 6-ridged. There are numerous ovules on axile placentæ. In the middle of the ovary the ovules lie perpendicular to the central axis, but at the two ends they are variously inclined to it. There is a short style with a triangular stylar canal, which is lined by radially elongated, richly protoplasmic cells.

In both species, the ovules are bitegmic and anatropous. The micropyles of the fertilisable ovules are formed only by the outer integument. Though the inner integument is demarcated simultaneously with the outer, it shows tardy growth and covers about a third of the nucellus in *Corchorus acutangulus* (Fig. 52) and about ¾ in *Triumfetta rhomboidea* (Fig. 54). After fertilisation, the inner integument also grows up and closes to form the micropyle, which then has the zig-zag form characteristic of the Malvales (Figs. 51 and 56). Except in the region of the micropyle, the outer integument is 2-cells thick and the inner is uniformly 3 cells thick. As in members of Sterculiaceæ (Rao, 1950, 1951), the outer layer of the outer integument and the innermost layer of the inner integument lose their cytoplasm and get filled with deep staining contents (Fig. 40). In microtomed sections of mature ovules, an air space is seen between the integuments, either all round or on the side away from the funicle.

The nucellus is straight and massive. In *Triumfetta*, the 2 or 3 layers of parietal tissue formed by the primary parietal cell get crushed by the 2- or 4-nucleate embryo-sac and the nucellar epidermis both at the sides and apex of the ovule undergoes periclinal divisions to form 5–10 layers of cells. These form the bulk of the nucellus in this species (Fig. 40). The post-fertilisation growth of the ovule is due to further divisions in these cells. The apical part of the nucellus shows a beak-like protuberance which projects into the micropyle. Later, after the inner integument closes up, this appears as a small wedge in the lower part of the micropyle (Figs. 54 and 56). The cells of the nucellus


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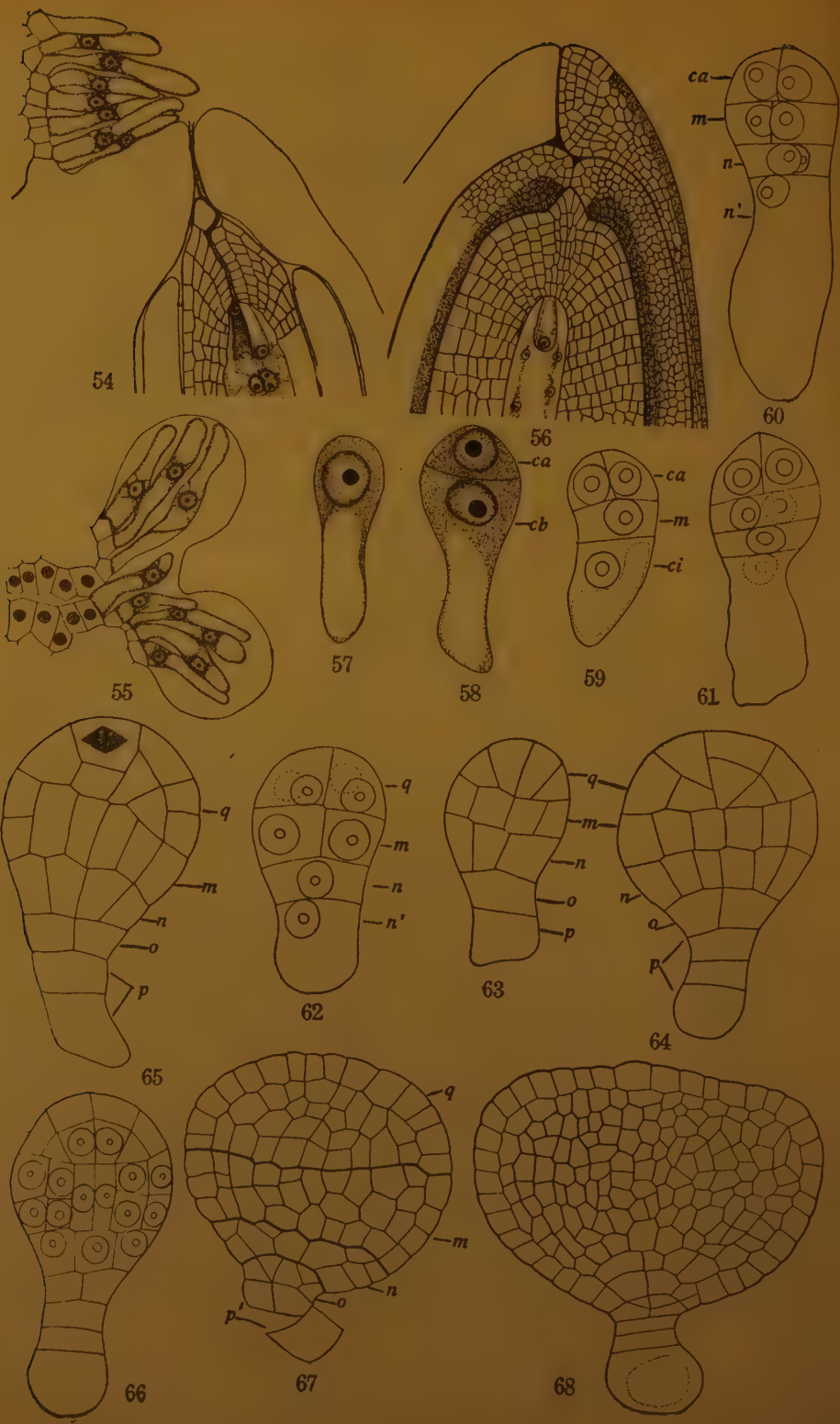
FIGS. 41–53. *Corchorus acutangulus.*—Fig. 41. T. S. of a loculus of the ovary showing ovule primordia. Fig. 42. Young ovule with multicellular archesporium. Figs. 43 and 44. Ovules with 2 functional archesporial cells. Figs. 45 and 47. Formation of parietal tissue. Fig. 46. Formation of linear tetrad. Figs. 48 and 49. T-shaped tetrads. Fig. 50. Young embryo sac. Fig. 51. Mature ovule with fertilised egg. Fig. 52. L. S. fertilisable ovule. Fig. 53. Fertilisable embryo sac. Fig. 41, × 325; Figs. 42–50, × 700; Fig. 51, × 45; Fig. 52, × 425; Fig. 53, × 700

around the antipodal end of the embryo-sac become thick-walled as in several members of Sterculiaceæ. A group of cells in the chalazal region of the fertilisable ovules stains deeply due to the presence of tannin, a feature met with in several Malvales. The chalaza shows a conical protuberance. In *Corchorus acutangulus*, the nucellus is less extensive (Fig. 52). In the fertilisable ovule there are only 2–3 layers of cells above and on the sides of the embryo-sac. The apical part of the nucellus is slightly bent. As the embryo-sac does not show a corresponding curvature, the micropyle is not situated directly above the egg apparatus but to one side. The zone of tannin-bearing cells in the chalaza becomes conspicuous in ovules only after fertilisation (Fig. 51).

MEGASPOROGENESIS AND FEMALE GAMETOPHYTE

When the archesporium differentiates in the ovule, the integument initials are not demarcated (Figs. 28–30). The primary archesporium is multicellular to start with in both species, consisting of both hypodermal and sub-hypodermal cells (Figs. 28 and 42). In *Corchorus acutangulus*, only one cell continues to function, while the rest merge into the nucellus. Only occasionally two collaterally placed cells were seen to function till the formation of parietal cells (Figs. 43 and 44), as also reported in *Corchorus* species studied by Banerji (1933 *b*) and *Tilia tomentosa* (Stenar, 1925). In *Triumfetta rhomboidea*, however, more than one archesporial cell function as a rule. In several ovule primordia, the cells of the nucellus are seen to be arranged in four more or less regular rows. The four hypodermal cells function as archesporial cells (Figs. 28–31). They divide forming the primary parietal cells to the outside and the megaspore-mother cells to the inside. This division does not occur simultaneously in all the archesporial cells of an ovule (Fig. 29). In case the arrangement of the nucellus cells is not so regular, a group of hypodermal and sub-hypodermal cells which may be more than 4 in number function as the archesporium (Fig. 32). Only the hypodermal cells cut off primary parietal cells, while the deep seated cells function directly as the megaspore-mother cells (Fig. 32). With further growth, the megaspore-mother cells attain elongated and tapering form, with broad micropylar and narrow chalazal ends. Their nuclei are situated close to the micropylar ends. Their chalazal ends converge on one or two nucellus cells placed below, which are larger than the rest and show large nuclei and vacuolated cytoplasm. These cells probably function as the nutritive cells. Thus the structure of the sporogenous cells is closely similar to that in *Pterospermum heyneanum* (Rao, 1949), though the number of sporogenous cells in *Triumfetta* is smaller. Only rarely an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FIGS. 54–68. *Triumfetta rhomboidea*.—Fig. 54. L S. Ovule showing pollen tube and obturator. Fig. 55. T. S. Ovary at the top showing obturator. Fig. 56. Upper part of fertilised ovule showing the micropyle, integuments and the nucellar beak. Fig. 57–68. Stages in the development of the embryo. Figs. 54 and 55, × 325; Fig. 56, × 135; Figs. 57–68, × 700.

ovule was seen to contain a single megaspore mother cell (Fig. 34). By the time the megaspore mother cell is ready to divide, there are 5 layers of parietal tissue above it, including an epidermal cap of 3 layers (Fig. 33).

In *Corchorus acutangulus*, T-shaped tetrads were noticed occasionally (Figs. 48 and 49), but in *Triumfetta* all tetrads were found to be linear (Figs. 34–37). In the latter species, all the megaspore-mother cells may form tetrads or some of them may degenerate earlier. In any case, only the chalazal megaspore of a tetrad functions and the rest degenerate quickly. Only in a single instance, two 1-nucleate embryo-sacs were seen in one ovule (Fig. 37). The functional megaspore enlarges, crushing the nutritive cell placed below it. Soon afterwards, the group of cells surrounding the embryo-sac become thick-walled.

The embryo-sac, which is organised after 3 free nuclear divisions of the megaspore nucleus, shows normal features. Cell walls are organised earlier around the antipodals. The synergids are hooked and show large vacuoles at their chalazal ends. The two polar nuclei remain separate till the time of fertilisation and the cytoplasm of the embryo-sac shows starch grains. The three antipodals degenerate early (Fig. 39).

FERTILISATION

The style in *Triumfetta rhomboidea* does not show any stylar canal. The pollen tube passes in an endotropic manner and reaches the base of the style. From the septum at the top of the ovary, several unicellular hairs arise and stretch towards the micropyles of the ovules, though they do not penetrate them. These bridge the gap between the top of the ovary and the micropyle and serve as an obturator (Figs. 54 and 55). Such a hairy obturator is seen in Thymelæaceæ (Venkateswarlu, 1945), and *Pilea annulata* (Fagerlind, 1941). Stenar (1925) also reported the occurrence of multicellular hairs below the micropyles of the ovules in *Modiola caroliniana*, which become especially prominent in fertilisable ovaries, but he did not mention their function. The pollen tube penetrates the ovule in a porogamous manner and enters a synergid which henceforth stains deeply. Later this bursts to liberate the male nuclei, both of which show distinct nucleoli. One male nucleus fuses with one of the polar nuclei. Later a 3 *n* primary endosperm nucleus is formed by the fusion of the second polar nucleus as was also found in *Corchorus olitorius* (Banerji, 1933 *b*), *Waltheria indica* (Rao, 1950) and *Melochia corchorifolia* (Rao, 1951). The pollen tubes are ephemeral and no trace of them is left by the time a few endosperm nuclei are formed.

ENDOSPERM

The primary endosperm nucleus divides without any period of rest. The endosperm is nuclear to start with and becomes cellular by

the time the embryo shows cotyledon primordia. Cell wall formation commences at the micropylar pole and ultimately the whole endosperm gets cut up into uninucleate cells which get packed with starch grains.

Embryo

An ovule with a fertilisable embryo-sac in *Triumfetta rhomboidea* measures 500 μ while one with fertilised egg about to divide, measures 950 μ. So it is evident that the fertilised egg rests for some days though the exact period could not be ascertained. In *Corchorus olitorius* Banerji (1933 *b*) found that the first division of the fertilised egg occurs 14–15 days after fertilisation. The enlargement of the embryo-sac is also quite marked during this period; it grows from 90 μ to 300 μ, and becomes tubular.

The development of the embryo conforms to the Urtica variation of the Asterad Type (Johansen, 1950) (Figs. 57–68). After fertilisation, the egg becomes much elongated and retains the basal vacuole. It divides transversely, the spindle being laid nearer to the distal end of the cell. So the basal cell (*cb*) is elongated and provided with vacuolated cytoplasm and the terminal cell (*ca*) is smaller and densely protoplasmic. *Ca* undergoes two longitudinal divisions in intersecting planes and gives rise to the quadrants (*q*). It is destined to give rise to the cotyledons and stem tip. *Cb* undergoes a transverse division, forming *m* and *ci*. *M* now undergoes divisions in the same manner as *ca* and forms quadrants, which develop into the hypocotyl. *Ci* divides transversely forming *n* and *n'*. *N* gives rise to the root tip after undergoing divisions similar to those in *m*. *N'* divides transversely forming *o* and *p*. *O* behaves as the hypophysis and fills out the dermatogen of the root tip and root cap, while *p* forms the suspensor. So the stem tip and the cotyledons are formed by *ca* and the rest of the embryo by *cb*. To form the octants, the quadrants *q* of the terminal tier divide in an inclined manner characteristic of the Urtica variation of the Asterad Type. The suspensor remains uniseriate till the cotyledon primordia are differentiated. The mature embryo is large and straight with foliaceous cotyledons and well developed stem tip and root tip.

The seed is endospermic but without perisperm.

Seed Coats

The formation and structure of the seed coats are closely similar to those of Sterculiaceæ. After fertilisation, the outer integument becomes 3-layered, but remains as a membraneous testa. The inner integument becomes 8–9 cells thick. The outermost forms the palisade layer with thick-walled radially elongated cells. The subepidermal layer consists of enlarged cells, which show deep staining contents. The innermost layer consists of tangentially flattened cells which also show deep staining contents. In the seed, the tegmen consists of these three layers while the median parenchymatous layers get crushed and absorbed.

Discussion

The families included in the order Malvales show several common embryological features like 2-nucleate mature pollen grains, bitegmi

anatropous ovule with a zig-zag micropyle formed by both the integuments, massive nucellus, formation of epidermal cap, zone of tannin-bearing cells in the chalaza, structure and development of the embryo-sac according to the *normal*-type, endotropic growth of the pollen tube, porogamous entry of the pollen tube, fusion of the polar nuclei only at the time of fertilisation, nuclear endosperm which becomes cellular only at a late stage in the development of the embryo, embryogeny according to the Urtica variation of the Asterad Type (excepting *Muntingia calabura* of Elæocarpaceæ (Rao, 1951*a*), which follows the Onagrad type), large embryo and absence of perisperm in the seed, structure and development of the seed coats, etc. So many common embryological features show that the Order is a very homogeneous one. A close comparison of the characters shows that Tiliaceæ resembles Sterculiaceæ more closely than Malvaceæ. Among these can be mentioned the secretory type of anther tapetum, secondary increase in the number of microspore mother cells, smooth-walled pollen grains with 3 germinal furrows and 3 germ pores, hooked synergids, presence of starch grains in the cytoplasm of the embryo-sac, presence of 3 ephemeral 1-nucleate antipodals, organisation of a socket of thick-walled cells around the antipodal end of the embryo-sac, straight embryo-sac, a chalazal outgrowth on the ovule and a short suspensor for the embryo.

Summary

Development of the male and female gametophytes has been studied in *Triumfetta rhomboidea* Jacq. and *Corchorus acutangulus* L. and the embryogeny in the former species.

The organogeny of the flower of *Triumfetta rhomboidea* shows tendency towards the suppression of petals, which appear last.

The anther tapetum is of the secretory type. Microspore tetrads are mostly tetrahedral and cytokinesis occurs by furrowing. The chromosome number in *Triumfetta rhomboidea* is $2n = 48$ and $n = 24$. The spindle formed in the division of the microspore nucleus is asymmetrical with pointed vegetative and rounded generative poles. Mature pollen grains are ellipsoidal, 2-nucleate, with smooth exine which shows 3 fusiform germinal furrows with a pore in the middle of each.

The ovules are bitegmic, anatropous and crassi-nucellate. The micropyle is formed only by the outer integument in the fertilisable ovule, but after fertilisation, the inner integument also grows up and forms a part of the micropyle. The archesporium is multicellular. In *Corchorus acutangulus*, only one cell functions while in *Triumfetta rhomboidea*, 4–5 cells function till the formation of tetrads. A few T-shaped tetrads are seen in *Corchorus acutangulus*. The embryo-sac is formed according to the *normal*-type.

Entry of the pollen tube is porogamous and is facilitated by the presence of hairy obturator in *Triumfetta rhomboidea*. Endosperm is nuclear to start with. The development of the embryo conforms to the Urtica variation of the Asterad Type. Mature seed is endospermic and shows a large and straight embryo.

The outer integument forms only a membraneous testa. The palisade layer of the seed coat is derived from the outermost layer of the inner integument.

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Literature

Banerji, I. 1933 *a*. The development of flower and pollen in jute. The Ind. Jour. Agri. Sci. 3: 116–125.

———————. 1933 *b*. Development of the embryo-sac and fertilisation in jute. Jour. Ind. Bot. Soc. 11: 228–239.

Brumfield, R. T. 1941. Asymmetrical spindles in the first microspore division of certain angiosperms. Amer. Jour. Bot. 28: 713–722.

Fagerlind, F. 1944. Die samenbildung und die Zytologie bei agamospermischen und sexuuellen Arten von *Elatostema* und einigen nahestehenden Gattungen nebst Beleuchtung einiger damit zusammenhangender probleme. K. Svenska Vet. Akad. Haldl. III 21 (4) : 1–130.

Hagerup, O. 1938. A peculiar asymmetrical mitosis in *Orchis*. Hereditas. 24: 94–96.

Johansen, D. A. 1950. Plant Embryology. Chronica Botanica. Waltham. Mass.

Juel, H. O. 1915. Untersuchangen uber die Auflosung der Tapetenzellen in den Pollensacken der Angiospermen. Jahrb. f. Wiss. Bot. 56: 337–364.

Rao, C. V. 1949. Contributions to the embryology of Sterculiaceæ—I. Jour. Ind. Bot. Soc. 28: 180–197.

———————. 1950. Contributions to the embryology of Sterculiaceæ—II. *Waltheria indica* L. Ibid. 29: 163–176.

———————. 1951. Contributions to the embryology of Sterculiaceæ—III. *Melochia corchorifolia* L. Ibid. 30: 122–131.

———————. 1951 *a*. Life-history of *Muntingia calabura* L. Curr. Sci. 20: 47–48.

Rao, L. N. 1941. Cytology of *Hibiscus trionum* L. New Phytol. 40: 326–335.

Schnarf, K. 1931. Vergleichende Embryologie der Angiospermen. Berlin.

Sommer, B. S. 1929. Uber Entwicklungshemmungen bei Samenanlagen. Flora. 124: 63–94.

Souèges, R. 1941. Développement de l' embryon chez le *Tilia platyphyllos*. Scop. Comt. Rend. Acad. Sci. 212: 993–1000.

Stenar, H. 1925. Embryologische Studein. I. Zur embryologie einiger Columniferen. Akad. Abh. Uppsala.

Strasburger, E. 1884. Neuere Untersuchungen uber den Befruchtungsvorgang bei den Phanerogamen als Grundlage fur eine Theorie der Zeugung. Jena.

Venkateswarlu, J. 1945. Embryological studies in the Thymelæaceæ—I. Jour. Ind. Bot. Soc. 24: 45–66.

Warming, E. 1878. De l' ovule. Ann. Sci. Nat. Bot. ser. 6. 5: 177–266.

Wallisch, R. 1930. Die chromosomenverhaltnisse bei *Tilia platyphyllos*, *T. cordata* und *T. argentea*. Öst. Bot. Zeitschr. 79: 97–106.

STUDIES IN CROP PHYSIOLOGY

Effect of Nitrogen, Phosphorus and Potassium on Growth and Pigment Content of Tobacco

By K. N. Lal and R. S. Tyagi

Plant Physiology Laboratory, College of Agriculture, Banaras Hindu University

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Introduction

This paper elucidates the effect of nitrogen, phosphorus and potassium on growth characters, pigment content and yield of tobacco grown in soil and sand cultures. Results presented are the outcome of two series of investigations on (i) the measurable effects of these nutrients in soil cultures; and (ii) deficiency effects in sand nutrient media. The responses in both these cases are analysed in terms of several growth characters, *e.g.*, height, leaf size, leaf number, leaf yield, curing properties, duration of flowering and pigment content of leaves. These were taken up with the dual purpose of ascertaining the best combination of N, P and K for yield and quality and to elucidate the manner in which growth of tobacco is likely to be affected in presence or absence of these ingredients. They portray the effects noted in pot cultures and depict how a systematic investigation of the problem is essential to a thorough analysis of the response of nutrients.

Other investigators have shown that nitrogen increases yield, leaf area, and leaf to stalk ratio (Garner, *et al.*, 1934 and 1939). Better growth of shoot and earliness in flowering (Garner, *et al.*, 1934) are also recorded alongside increases in water content and total and protein nitrogen (Armstrong and Albert, 1931). Further inorganic forms of nitrogen yield better than organic ones. Deficiency causes frenching disease with light green colour of foliage, yellowing and firing of lower leaves (Garner, 1934; MacMurtrey, 1934) and sluggish stomata (Desai, 1937).

With potassium, yield of tobacco is not so much affected as quality of leaves. Iron content is promoted and resistance to mosaic is induced (Spencer, 1935). Its effects are largely controlled by calcium and nitrogen. Deficiency symptoms include mottling and downward curling of leaves, crumping of leaf tips and margins (Haas and Klotz, 1931) and chlorosis (MacMurtrey, 1934) and formation of bacterial leaf spots.

Phosphorus improves leaf yield, dry weight and quality of foliage and induces resistance to mosaic (Spencer, 1935). It is mostly assimilated during later periods of life-cycle. Its absence results in dark green colour, delayed maturity and firing of leaves with spots of different colours (Desai, 1937; MacMurtrey, 1934) on the lamina,

Iron content and pH of the medium largely determine the intensity of these spots.

To what extent these and other symptoms develop in the local soil is elucidated in the following pages.

Experimentation

Harissons's tobacco seedlings were raised in November 1944 and planted three in each pot filled either with sand (80 lbs.) or sandy loam soil (72 lbs.). Plants were subsequently thinned down to one per pot. Hoeing, weeding and irrigations were done at regular intervals. Nutrients were added as indicated in the two experiments below:

Experiment 1.—Eight combinations of O, N, P, K, NP, NK, PK, and NPK were tried at corresponding levels of 150 ppm. N (sulphate of ammonia), 75 ppm. P_2O_5 (superphosphate) and 75 ppm. K_2O (sulphate of potash) all calculated on weight of soil basis. One hundred and twenty cultures including eight treatments, three replicates and five pots per replication were maintained. Nutrients were added in three equal doses at 15, 35, and 55 days after transplanting.

Experiment 2.—Four different conditions of nutrient supply were maintained with Hoagland's nutrient solution supplied in two instalments $\frac{2}{3}$ after thinning and remaining $\frac{1}{3}$ a fortnight later making up a total of six litres of nutrient solution. Drainage holes were closed at the base. Adequate irrigation was given in regulated quantity with tap water. But for the small contamination from tap water and the sand used, the cultures in general depicted four conditions of nutrition, *viz.*, (i) complete nutrition, (ii) nitrogen deficiency, (iii) phosphorus deficiency and (iv) potassium deficiency. Boron, zinc, copper, molybdenum, manganese and iron were also supplied in small quantity along with the macro-element solution of Hoagland as indicated elsewhere (Singh, 1940). In all, forty cultures for each of the above conditions of nutrition, were maintained for purposes of present study.

Plant Characters.—Various growth characters were recorded at successive stages or at harvest. Inflorescence appearance was noted on each day commencing flowering. Curing of leaves on a laboratory scale was done in an electric oven with an initial temperature of 80° F. gradually rising to 95° F. during 1-3 hours and maintained at this temperature for 18 hours. Yellow leaves were later transferred to another oven at 105° F., the temperature of which was subsequently raised to 120° F. within an hour. When yellow colour was fixed, temperature was further raised to 160° F. till the entire lamina dried up uniformly. Finally dried leaves were kept in a very humid chamber for softening. For estimation of leaf pigments, chlorophyll *a*, chlorophyll *b*, carotin and xanthophyll, a 5 gm. sample of fresh green leaf was taken and pigments extracted after the manner indicated elsewhere (Loomis and Shull, 1937). Concentration of individual pigments was estimated with Klett Colorimeter using Gutherie's standards for the yellow and green pigments. Pigment concentration was expressed in milligram per 10 gms. fresh weight of leaves.

Experimental Results

A. *Growth characters of tobacco in relation to N, P and K supply in soil*

Height of plants varied with age and nutrition. In general, height increased with advance in age. Till 70 days, none of main effects of fertilisers were significant. First order interactions P × K and N × P were both positively significant at 35 and 55 days respectively. Other effects were non-significant. At 85 days, nitrogen and phosphorus significantly increased height. At a later stage of 100 days, only nitrogen showed positively significant main effect. First order interactions N × P and N × K and higher order interaction N × P × K were all negatively significant at 85 and 100 days. Of the three nutrients, only nitrogen consistently showed tendency to increase height at all stages; the effects were significant only towards the end. Potash effects were continuously insignificant. Phosphorus was positively significant at one stage (85 days) only (Table I).

Table I

Mean height of main shoot as affected by fertilisers (Expt. I)

Age	N	P	K	NP	NK	PK	NPK	O	S. D. at 5%
35 days									
Mean height	4·66	4·66	3·00	3·33	3·50	4·33	5·33	4·33	..
Response ..	*0·50*	*2·17*	*−0·83*	*−1·16*	*2·50*	*4·50*	*2·17*	..	*3·72*
55 days									
Mean height	25·0	26·00	27·30	32·6	29·5	19·0	33·6	29·6	
Response ..	*20·2*	*−0·17*	*−3·83*	*23·8*	*14·5*	*−8·20*	*1·20*	..	*22·09*
70 days									
Mean height	47·6	42·6	43·3	55·0	48·6	41·0	44·6	48·6	..
Response ..	*20·3*	*−5·0*	*−15·6*	*11·6*	*−2·3*	*−7·6*	*−15·0*	..	*37·1*
85 days									
Mean height	85·3	65·0	62·7	90·0	80·0	84·0	74·7	56·6	..
Response ..	*61·6*	*29·0*	*4·3*	*−19·6*	*−35·0*	*−4·3*	*−23·0*	..	*12·78*
100 days									
Mean height	116·0	70·6	70·3	126·0	116·0	100·0	95·0	67·6	..
Response ..	*144·3*	*21·6*	*1·0*	*−43·0*	*−63·0*	*−4·3*	*−54·6*	..	*34·75*

Leaf number was not markedly affected by nutrients upto 70 days. At subsequent periods, nitrogen consistently increased leaf number. Significant effects were noted at 70, 85 and 100 days. Responses of P and K were insignificant (Table II). All first order interactions excepting N × K at 100 days, showed no marked response. Nitrogen affected this character prominently during later part of life-cycle.

Dry leaves were reduced significantly under nitrogen. Average life duration of individual leaves was prolonged more particularly after 70 days in life-cycle. Phosphorus had no effect. Potash reduced dry leaves at 70 days significantly (Table III).

TABLE II

Mean number of green leaves as affected by fertilisers (Expt. I)

Age	N	P	K	NP	NK	PK	NPK	O	S.D. at 5%
35 days									
Leaf number	8·3	9·7	8·0	8·0	9·7	9·3	10·0	8·3	..
Response ..	*0·3*	*3·0*	*2·3*	*1·0*	*3·6*	*1·0*	*−1·0*	..	*6·9*
55 days									
Leaf number	13·7	14·0	14·0	15·0	14·0	13·3	14·6	14·6	..
Response ..	*1·3*	*0·7*	*−1·3*	*3·3*	*−1·3*	*−0·6*	*−0·6*		*8·33*
70 days									
Leaf number	18·0	12·3	14·3	16·7	17·0	14·0	18·0	14·6	..
Response ..	*31·0*	*−3·0*	*1·7*	*2·3*	*−1·0*	*4·3*	*−0·3*	..	*10·64*
85 days									
Leaf number	22·6	18·3	16·3	25·0	25·0	20·0	24·6	15·6	..
Response ..	*27·0*	*8·3*	*4·3*	*−4·3*	*−0·3*	*−1·7*	*−3·7*	..	*9·61*
100 days									
Leaf number	29·0	17·0	21·3	27·0	23·0	23·7	26·7	15·7	..
Response ..	*28·0*	*5·3*	*6·0*	*−2·0*	*−18·6*	*6·7*	*4·7*	..	*7·07*

S.D. = Significant difference

TABLE III

Number of dry leaves as affected by fertilisers (Expt. I)

Age	N	P	K	NP	NK	PK	NPK	O	S.D. at 5%
70 days									
Dry leaf No.	3·7	5·0	3·7	4·0	3·0	3·7	2·7	4·3	..
Response	*−3·3*	*0·7*	*−3·7*	*−0·7*	*0·0*	*−1·3*	*0·0*	..	*2·93*
85 days									
Dry leaf No.	4·0	5·3	6·3	4·0	4·0	6·3	4·7	8·3	..
Response	*−9·7*	*−2·3*	*−0·3*	*3·7*	*1·7*	*3·7*	*−2·3*	..	*3·62*
100 days									
Dry leaf No.	6·0	9·3	9·7	5·7	6·0	8·7	5·7	9·3	..
Response	−13·7	−1·7	−0·3	0·3	0·3	−1·0	1·0	..	4·44

S.D. = Standard difference

Width of leaves was improved by nitrogen at 55, 70, 85, and 100 days while phosphorus and potash showed significant effects at 70 days only. N × K and P × K interactions reduced width of foliage at 70 and 85 days respectively. Other effects were insignificant (Table IV).

Length of leaf was better under nitrogen feeding at all stages beyond 35 days. Potash improved this at 70 days only. Phosphorus effects were consistently insignificant. P × K interaction showed negative significance at 85 days (Table V).

TABLE IV

Breadth of leaves as affected by fertilisers (Expt. I)

Age	N	P	K	NP	NK	PK	NPK	O	S.D. at 5%
35 days									
Breadth ..	4·7	4·7	4·5	4·5	4·4	4·6	4·0	4·8	..
Response ..	*−0·0*	*−1·5*	*−0·9*	*0·5*	*−0·7*	*0·4*	*−0·7*	..	*2·87*
55 days									
Breadth ..	11·1	8·9	9·9	10·6	12·3	8·4	10·6	8·8	..
Response ..	*9·3*	*−4·4*	*2·6*	*−0·1*	*−0·2*	*−0·2*	*−0·3*	..	*4·61*
70 days									
Breadth ..	12·4	9·7	11·3	13·7	12·9	11·4	13·1	8·5	..
Response ..	*11·2*	*2·7*	*4·5*	*0·1*	*4·8*	*−2·2*	*−0·03*	..	*2·44*
85 days									
Breadth ..	13·1	11·6	11·7	15·2	14·9	9·6	15·3	8·2	..
Response ..	17·0	3·8	3·4	1·1	0·4	−7·2	3·6	..	4·43
100 days									
Breadth ..	14·2	9·7	10·1	16·0	16·9	15·4	9·1	9·2	..
Response ..	24·4	−0·2	2·5	0·8	1·8	−4·8	−1·8	..	3·08

S.D. = Significant difference

TABLE V

Length of leaves as affected by fertilisers (Expt. I)

Age	N	P	K	NP	NK	PK	NPK	O	S.D. at 5%
35 days									
Length ..	8·6	7·9	8·6	7·5	8·7	8·0	8·3	9·1	..
Response ..	*−0·4*	*−3·3*	*0·5*	*0·2*	*1·2*	*1·2*	*0·07*	..	*4·65*
55 days									
Length ..	20·5	18·5	19·5	19·9	22·2	17·6	21·9	19·7	..
Response ..	*9·4*	*−3·8*	*2·5*	*2·3*	*4·9*	*−0·4*	*1·0*	..	*7·96*
70 days									
Length ..	25·8	21·4	23·5	30·0	27·0	24·8	26·9	20·3	..
Response ..	*16·1*	*2·9*	*8·4*	*−1·3*	*−5·4*	*−0·9*	*−0·8*	..	*8·30*
85 days									
Length ..	26·4	25·1	24·8	29·9	30·0	21·5	28·8	19·8	..
Response ..	*23·8*	*4·3*	*3·9*	*−0·4*	*1·2*	*−13·2*	*3·9*	..	*9·23*
100 days									
Length ..	29·9	20·4	21·7	32·6	31·6	20·5	29·5	22·7	..
Response ..	*38·3*	*−2·9*	*−2·4*	*4·1*	*−0·6*	*−3·6*	*−5·8*	..	*6·68*

S.D. = Standard difference

In P and K fed plants, buds appeared 82 days after planting; duration of flowering extended over 13 days. In control, buds appeared after 86 days and flowering extended over 10 days only In presence of nitrogen, flowering was delayed to 95 days but entire population flowered within eight days (Table VI). Chlorophyll *a* and chlorophyll *b* contents were significantly improved under nitrogen feeding at 70 days in life-cycle. Phosphorus on the other hand, improved

TABLE VI

Number of plants flowering on different dates in different fertiliser treatments (Expt. I)

Age in days	N	NP	NK	NPK	P	K	PK	Control
82	..	..	..	..	2	3	1	0
84	..	..	..	..	1	2	1	0
86	..	..	..	..	4	4	4	1
88	..	..	..	..	2	0	1	2
90	..	..	..	..	4	3	4	2
92	..	..	..	..	1	1	3	5
93	..	..	..	..	1	2	0	1
95	2	1	0	0	0	0	1	2
97	1	4	2	3	0	0	0	2
99	5	2	2	1	..	..	..	..
101	4	6	7	8	..	..	..	..
102	3	2	4	2	..	..	..	..
105	0	0	0	1	..	..	..	..
Total ..	15	15	15	15	15	15	15	15

chlorophyll *a* only. Both the green pigments were reduced under potassium. Potassium also reduced carotin content while other fertiliser ingredients were ineffective. Carotin was also improved under N×P×K interaction. Similarly P×K interactions improved xanthophyll. Reduction in xanthophyll was also observed under N×P and N×P×K interactions (Table VII).

Of all the pigment ratios, nitrogen markedly improved total green/total yellow, chlorophyll *a*/carotin, chlorophyll *a*/xanthophyll, chlorophyll *b*/carotin and chlorophyll *b*/xanthophyll ratios. This indicated the outstanding effect of N in improving total and individual green pigments more than total yellow or individual yellow pigments. Phosphorus improved chlorophyll *a*/xanthophyll ratio only. Potassium on the contrary, showed significant increases in chlorophyll *a*/chlorophyll *b* ratio and significant reduction in chlorophyll *b*/xanthophyll ratio. Further reduction in chlorophyll *a*/carotin and xanthophyll/carotin ratios were noted under N×P and N×P×K interactions. On chlorophyll *a*/xanthophyll, N×P showed significant decrease; P×K also indicated marked decrease. Similar reduction in chlorophyll *b*/carotin ratio under N × P × K interaction was also observed (Table VII).

At harvest, nitrogen was the only nutrient that improved leaf, stem and weight of roots. Phosphorus tended to improve leaf and root weight and reduce weight of stem but effects were insignificant. Similarly potash effects were insignificant (Table VIII). None of the first or higher order interactions exhibited significant effect. High yield was invariably recorded in cultures where nitrogen was available singly or in combination with other nutrients. NP cultures proved best from yield point of view.

TABLE VII

Pigment content and pigment ratios as affected by fertilisers (Expt. I)

Pigment	N	P	K	NP	NK	PK	NPK	O	S.D. at 5%
Chl. *a* ..	9·75	8·12	6·82	9·94	9·26	9·57	7·54	7·48	..
Response ..	*8·56*	*1·85*	*−2·07*	*−0·87*	*0·39*	*0·18*	*0·03*	..	*1·27*
Chl. *b* ..	4·52	3·67	2·99	4·59	4·23	4·30	3·30	4·23	..
Response ..	*3·53*	*−0·11*	*−2·19*	*0·38*	*1·02*	*0·86*	*−0·86*	..	*1·06*
Carotin ..	1·24	1·15	1·17	1·07	1·93	1·18	..	1·04	
Response ..	*0·18*	*−0·09*	*−0·32*	*0·22*	*0·06*	*0·02*	*0·81*	..	*0·89*
Xanthophyll ..	1·80	1·62	1·48	1·61	1·68	1·52	1·86	1·76	..
Response ..	*−0·12*	*−0·12*	*−0·26*	*−0·58*	*−0·19*	*0·56*	*−0·48*	..	*0·36*
Green yellow pigments	4·67	4·24	3·69	5·44	5·17	5·14	3·83	4·17	..
Response ..	*13·15*	*1·04*	*−0·59*	*0·40*	*0·99*	*−0·63*	*−0·96*	..	*1·07*
Chl. *a*/Chl. *b* ratio	2·17	2·22	2·23	2·16	2·19	2·22	2·28	1·78	..
Response ..	*0·16*	*0·48*	*0·65*	*−0·43*	*−0·51*	*−0·40*	*0·47*	..	*0·59*
Chlorophyll *a*/ carotin ratio	7·90	7·04	5·82	9·30	9·91	8·1	8·40	7·20	..
Response ..	*6·78*	*2·10*	*0·76*	*−2·80*	*0·76*	*−0·46*	*−6·01*	..	*2·73*
Chlorophyll *a*/ Xanthophyll	5·38	5·01	4·59	6·22	5·55	6·29	4·08	4·23	..
Response ..	*5·53*	*1·85*	*−0·32*	*−1·29*	*0·81*	*−1·38*	*1·19*	..	*1·24*
Chlorophyll *b*/ carotin	3·64	3·19	2·25	4·32	4·52	3·65	3·69	4·06	..
Response ..	*2·64*	*0·09*	*−0·80*	*0·47*	*1·23*	*0·45*	*−3·56*	..	*1·33*
Chlorophyll *b*/ Xanthophyll	2·99	2·26	2·01	2·87	2·53	2·83	1·77	2·39	..
Response ..	*2·78*	*−0·23*	*−1·38*	*0·55*	*0·36*	*0·31*	*0·53*	..	*1·18*
Xanthophyll/ carotin	1·46	1·37	1·32	1·53	1·79	1·28	2·07	1·69	..
Response ..	*−0·40*	*−0·01*	*0·42*	*−0·88*	*−0·23*	*0·52*	*−1·64*	..	*0·60*

S.D. = Standard difference

TABLE VIII

Weight of component parts at harvest as affected by fertilisers (Expt. I)

Plant part	N	P	K	NP	NK	PK	NPK	O	S.D. at 5%
Leaf weight ..	346·0	99·6	104·0	378·3	319·2	81·3	363·0	106·5	..
Response ..	*1014·0*	*47·0*	*− 63·0*	*106·0*	*−21·3*	*−4·3*	*27·3*	..	*157·0*
Stem weight ..	242·0	77·3	78·3	274·3	238·0	194·3	72·0	99·2	..
Response ..	*625·2*	*−36·2*	*−113·3*	*13·5*	*−54·5*	*−57·2*	*−94·8*	..	*146·2*
Root weight ..	71·2	41·7	36·2	79·6	69·3	75·3	40·6	37·2	..
Response ..	140·6	22·7	− 9·0	4·7	−5·0	−1·7	− 1·7	..	37·1

Nitrogen delayed maturity and prevented leaves from developing the characteristic yellowish colour. In P and K cultures finest quality of colour was developed. During curing O, P, K and PK fed plants acquired a bright golden yellow colour after 36 hours. This was satisfactorily fixed up within eight hours. N, NP, NK and NPK cultures showed poor development of yellow colour. Leaves turned brownish during colour fixation indicating poor curing quality. Relatively, PK treated plants showed best development of colour followed by K and P treated ones.

B. *Effect of nitrogen, potassium and phosphorus deficiencies on growth characters*

Complete nutrition plants were consistently better in height than deficient plants. Reductions were significant at 70 days when the order of effect was C, [−K, −P], and −N. This response was maintained throughout the remaining life-cycle (Table IX). Leaf number improved under complete nutrition at 70 days. At all stages subsequent to this nitrogen deficiency lowered leaf number significantly while differences between −P and −K were insignificant (Table X). Nutrient deficiency had no marked effect on dry leaves on shoot (Table XI).

TABLE IX

Height of plant as affected by various conditions of nutrition (Expt. I)

Age in days	C	−N	−P	−K	S.D. at 5%	Ratio of Variance
35	3·20	2·56	2·75	2·45	..	1·14
55	19·87	8·62	12·00	11·50	3·52	15·69
70	56·27	20·50	39·71	40·00	4·81	76·00
85	112·50	29·00	51·00	80·62	12·24	73·94
100	139·00	42·87	84·00	108·00	11·54	104·17

Value of F at 5 per cent. = 8·64

TABLE X. *Number of green leaves on plant under different conditions of nutrition (Expt. II)*

Age in days	C	−N	−P	−K	S.D. at 5%	Ratio of Variance
35	10·3	9·4	9·1	8·3	..	4·7
55	14·0	13·1	13·0	13·0	..	0·62
70	16·8	12·3	15·1	14·1	1·68	10·50
85	28·1	11·8	16·1	19·0	3·17	40·20
100	28·3	13·0	21·1	23·0	1·98	86·09

Value of F at 5 per cent. = 8·64

TABLE XI

Number of dry leaves on plant under different conditions of nutrition (Expt. II)

Age in days	C	−N	−P	−K	S.D. at 5%	Ratio of Variance
76	3·4	3·6	4·4	3·4	..	0·52
85	4·7	7·1	6·4	4·0	..	2·80
100	8·1	10·4	11·4	8·0	..	4·20

Value of F at 5 per cent. = 8·64

Complete nutrition produced plants with long leaves while nitrogen deficiency resulted in short leaves at all periods after 55 days. −P and −K cultures failed to show any significant difference in length of blade (Table XII). Leaf width was widest in complete nutrition

TABLE XII

Length of leaves under different conditions of nutrition (Expt. II)

Age in days	C	−N	−P	−K	S.D. at 5%	Ratio of Variance
35	9·6	7·4	8·2	7·6	..	..
55	20·2	12·6	16·5	15·1	2·55	13·04
70	31·7	16·8	25·5	26·7	2·46	62·09
85	51·5	20·6	24·4	26·6	3·59	9·34
100	31·0	19·7	26·9	26·2	2·08	40·95

Value of F at 5 per cent = 8·64

and smallest in −K even at 35 days; differences between −N and −P were not significant. Nitrogen deficiency effect only was significantly reduced width below the level recorded for C, −P, and −K at 55 days; at 70 days all deficiencies reduced leaf width in the order $-K, > -P > -N$. At later periods observed differences between −K and −P were insignificant (Table XIII).

Flowering was 100 per cent. under complete nutrition between 86 and 97 days. Under potash deficiency seven out of forty plants flowered and these too within a longer duration of 86 to 105 days. In nitrogen and phosphorus deficiencies none of the plants flowered. Suppressed vegetative growth in these cultures was followed by complete cessation of flowering. It appeared, therefore, that for normal initiation of reproduction, a certain minimum vegetative vigour

TABLE XIII

Width of leaves under different conditions of nutrition (Expt. II)

Age in days	C	−N	−P	−K	S.D. at 5%	Ratio of Variance
35	5·2	4·0	4·4	3·9	0·87	34·62
55	9·7	6·2	8·4	7·6	0·84	15·71
70	15·4	7·7	11·8	12·8	0·78	142·0
85	15·4	8·4	11·8	11·5	1·39	36·8
100	15·5	8·5	10·8	12·1	1·44	34·6

Value of F at 5 per cent. = 8·64

was absolutely necessary. This was not obtained in absence of nitrogen or phosphorus (Table XIV).

TABLE XIV

Number of plants flowering on different dates (Expt. II)

Age in days	C	−N	−P	−K
86	3	0	0	1
88	10	0	0	1
90	12	0	0	1
91	5	0	0	0
93	1	0	0	0
95	2	0	0	1
97	7	0	0	0
99	0	0	0	0
101	0	0	0	1
103	0	0	0	1
105	0	0	0	1
Total ..	40	0	0	7

Chlorophyll *a* and chlorophyll *a*/chlorophyll *b* ratio were significantly affected by the condition of nutrition provided to tobacco. Nitrogen significantly lowered chlorophyll *a* content. Phosphorus and potassium deficient plants failed to show any significant effect on this pigment. On chlorophyll *a*/chlorophyll *b* again, nitrogen deficiency was harmful. Phosphorus deficiency had no effect while potassium deficiency significantly raised this ratio (Table XV). above that of complete nutrition, nitrogen deficiency or phosphorus deficiency.

Nitrogen deficient plants were poorest in leaf, stem and root weight while phosphorus and potash deficiency produced plants with intermediate weight of leaf, stem and root.

TABLE XV

Effects of nitrogen, phosphorus and potassium deficiencies upon leaf pigments and pigment ratios

Pigments	Complete nutrition	−N	−P	−K	C.D. 5%
Chlorophyll *a* ..	8·35	5·32	8·43	7·49	1·31
Chlorophyll *b* ..	4·26	3·91	4·17	2·99	0·82
Carotin ..	0·89	0·96	1·70	1·10	0·22
Xanthophyll ..	1·81	1·63	1·53	1·37	..
Chlorophyll *a* / Chlorophyll *b* ..	1·96	1·35	2·03	2·54	0·38
Chlorophyll *a* / Carotin ..	9·39	5·67	7·90	6·85	2·02
Chlorophyll *a* / Xanthophyll ..	4·65	3·44	6·28	5·54	1·83
Chlorophyll *b* / Carotin ..	4·76	4·21	3·90	2·75	1·08
Chlorophyll *b* / Xanthophyll ..	2·36	2·51	2·81	2·23	..
Xanthophyll / Carotin ..	2·03	1·78	1·43	1·24	..
Total green / Total yellow ..	4·67	3·32	4·88	4·29	1·23

DISCUSSION

Observations under different conditions of nutrition point out the high efficiency of nitrogen in regulating the growth behaviour of tobacco. In soil, nitrogen significantly increases height, leaf number, leaf size and weight of all component parts. Flowering is delayed in all cultures where nitrogen is present alone or in combination with P or K or PK. Its useful effects on chlorophyll *a*, and chlorophyll *b* is established. Other pigment ratios, *e.g.*, green/yellow, chlorophyll *a*/carotin, chlorophyll *a*/xanthophyll, chlorophyll *b*/carotin and chlorophyll *b*/xanthophyll are also improved. This indicates that nitrogen affects the green pigments more than yellow. Higher green pigment is, however, a bar to good colour development during curing as a result of which nitrogen lowers the quality of tobacco.

Absence of nitrogen from the culture produces well marked reduction in vegetative vigour and fall in chlorophyll *a* and chlorophyll *a*/chlorophyll *b* ratio. Tyagi (1946) has presented a compre-

hensive picture of the results of other investigators who have also recorded useful effects of nitrogen on yield and water content. The deficiency effects are most prominent on width of leaves. Its availability ensures high yield but poor quality and therefore needs blending if good cigarette tobacco is desired.

Phosphorus plays a less significant role in that it increases height only during later stages and breadth of leaves during middle period of life-cycle. It has no significant effect upon plant weight. Chlorophyll *a* is increased in response to this ingredient; chlorophyll *a*/xanthophyll ratio is also high. Its deficiency lowers height, green leaves and leaf size below that of the control but not to that extent as noted for nitrogen-deficient plants. Flowering is completely suppressed in absence of phosphorus. No marked effect of P deficiency is noted on pigments. Phosphorus is claimed to affect quality more than yield and improves leaf area, leaf weight, flavour and dry weight. Both absence and excess of phosphorus delays shoot development. Its utility is greater during later periods when it produces its desirable effect on quality.

Potassium does not seem to affect any vegetative character except leaf size during middle portion of life-cycle. On plant weight it seems to have no effect. Its presence reduces chlorophyll *a*, chlorophyll *b*, and carotin. Chlorophyll *a*/chlorophyll *b* ratio is improved but chlorophyll *b*/xanthophyll is lowered in K fed plants. Deficiency of this element reduces vegetative characters slightly. Some inhitition in flowering is noted. Ratio of chlorophyll *a*/chlorophyll *b* is noted to be higher in its absence. Potassium also improves flavour while its affect on yield is determined by calcium. At lower calcium levels, application of potassium improves yield. Its utility is best during later periods of life-cycle (Tyagi, 1946).

Summary

This paper describes the effect of nitrogen, phosphorus and potassium on growth and pigment content of tobacco grown in pots filled with sand or sandy loam soil. In the first case four conditions of nutrition, *viz.*, complete nutrition, nitrogen deficiency, phosphorus deficiency and potassium deficiency were maintained with Hoagland's nutrient solution. In the second, eight combinations of N, P, and K were used with common fertilisers supplying these ingredients. Measurements of various characters were taken at successive stages of life-cycle. The following outstanding effects of these ingredients were noted in local soil:

Nitrogen.—Increases height, leaf number, leaf size and weight of component parts. Flowering is delayed. Improvement in chlorophyll *a* and chlorophyll *b* and larger proportion of green over yellow pigments are observed. Colour of cured leaf is poor.

Phosphorus.—Increases height during later period. Chlorophyll *a* is improved; a high chlorophyll *a*/xanthophyll ratio is maintained.

Potassium.—Affects leaf size during middle period of life-cycle. Chlorophyll *a*, chlorophyll *b* and carotin are significantly reduced.

Ratio chlorophyll *a*/chlorophyll *b* is improved while chlorophyll *a*/xanthophyll is lowered. Leaf colour during curing is improved.

In contrast to the above effects nitrogen deficiency reduces vegetative vigour, weight of component parts, chlorophyll *a*, chlorophyll *a*/chlorophyll *b* ratio, and inhibits flowering.

Phosphorus deficiency lowers all vegetative characters but to a lesser extent than nitrogen deficiency; flowering is completely suppressed.

Absence of potassium inhibits flowering partially with slight reduction in vegetative growth. Chlorophyll *a*/chlorophyll *b* ratio is noted to be higher.

Literature Cited

ARMSTRONG, G. M. AND ALBERT, W. B. 1931. Study of the cotton plant with special reference to its nitrogen content. Journ. Agric. Res. 42: 689–703.

DESAI, M. C. 1937. Effect of certain nutrient deficiencies on stomatal behaviour. Plant Physiol. 12: 253–283.

GARNER, W. W. *et al.* 1934. The nitrogen nutrition of tobacco. U.S.A. Department of Agriculture Tech. Bull. 414: 1–28.

————. 1939. Some aspects of the physiology and nutrition of tobacco. Journ. Amer. Soc. Agron. 31: 459–471.

HAAS, A. R. S. AND KLOTZ, L. J. 1931. Some anatomical and physiological changes in citrus produced by boron deficiencies. Hilgardia. 5: 175–186.

MACMURTREY, J. E. 1934. Fertiliser tests with tobacco. Md. Agric. Expt. Sta. Bull. 358: 255–290.

LOOMIS, W. E. AND SHULL, C. A. 1937. Methods in Plant Physiology. McGraw Hill Publications.

SINGH, B. N. Progress Report of Researches on the Physiology of Wheat and Cane. 1940. I.C.A.R. New Delhi.

SPENCER, E. L. 1935. Influence of phosphorus and potassium supply on host susceptibility to yellow tobacco mosaic infection. Phytopath. 25: 492–502.

TYAGI, R. S. 1946. Effect of N, P and K upon growth development and yield of tobacco. M Sc. Thesis, Banaras Hindu University.

THE AMOUNT OF FOLIAR ASH IN SAL (*SHOREA ROBUSTA* GAERTN.) TREES OF DIFFERENT QUALITY CLASSES IN INDIA

By G. S. Puri

Forest Research Institute, Dehra Dun

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In a recent study (Puri, 1950) it was found that miscellaneous forests of the Dehra Dun Valley, with little or no *Shorea robusta* occurred on neutral or alkaline soils, while pH values under Sal community were invariably much lower. Dehra Dun sal belongs to III all-India quality and soils in these forests, though acidic in comparison to miscellaneous forests of the same area, had slightly higher pH values than those recorded by Griffith and Gupta (1947) for good quality sal forests of the U.P. Though soil acidity seems to favour its growth, highly acidic conditions are unfavourable for sal. A critical comparison of my data with those of Griffith and Gupta and observations of other investigators (see Troup, 1921) seemed to show that *Shorea robusta* is probably a non-exacting species; and the available data for foliar ash and calcium in sal and a number of its associates in the Dun Valley forests (Puri and Gupta, 1950) supports the above contention. Leaves of Dehra Dun sal that belongs to III quality have 5·38% of ash and if *Shorea robusta* is physiologically non-exacting species foliar ash in good quality sal trees would probably be smaller than in poor quality trees. Any such relationship, however, may be disturbed by environmental factors for *Shorea robusta* has a very wide distribution in India occurring in different climatic conditions (see Champion, 1933) from Ganjam in the south to Ambala in the north-west and Assam in the east. It ascends from plains to altitudes of 5,000 ft. above sea level in the outer Himalayas and occupies a wide range of rocks and soils exhibiting large differences in quality of growth and development. Different quality classes of sal show large differences in height growth in a unit time and on the basis of this *sal* forests of the country have been divided into 4–5 all-India quality classes (Griffith and Sant Ram, 1943). Thus, I quality sal shows best growth in a unit time and a proportionate decrease in growth is indicated by II, III and IV quality classes. In addition to all-India qualities some States have their own quality classes which are different from the former. For example, C.P.* I quality sal corresponds to all India I/II; and C.P. II and III are equivalent to all-India II/III and III/IV quality classes respectively (Maitland, 1924).

* C.P. is now Madhya Pradesh.

The present investigation was undertaken to see if there are any differences in foliar ash of sal trees of different quality classes and whether these data throw any light on the non-exacting nature of this species. This approach to the problem of sal is original and so far as the author is aware no such work has been done before. However, Lutz and Chandler (1946, p. 147) have put together data of several American workers to show that foliar calcium in *Pinus strobus*, *Fagus grandifolia*, *Quercus alba*, *Quercus borealis*, *Acer saccharum*, *Liriodendron tulipifera* and *Tilia americana* is variable, but they do not relate these differences in foliar ash to quality of growth or development of tree species.

Leaves of I–IV quality classes of sal were procured with the kind co-operation of Provincial Silviculturists of Assam, Bengal, Bihar, Madhya Pradesh (C.P.), Orissa, Punjab and Uttar Pradesh (U.P.), to whom I am highly indebted. It will be difficult to thank individually by name numerous D.F.O.'s and Range Officers of these provinces who collected leaves for this study, but I wish to put on record my grateful thanks to all concerned.

1–2 lb. of full-grown mature leaves were collected from middle part of the crown of 9–10 dominant trees of a known quality class growing in an area of 100 square feet in the compartment sampled. One set of samples was collected between 5–10 November 1949, and the second set between 10–15 January 1950. The samples were received fresh, they were dried in sun, powdered in a grinding mill, sieved and ashed in an electric muffle furnace at 800° C. after determination of moisture content by keeping in a gas oven for 2 hours at temperature of 65° C.

The data have been divided into different foliar ash classes and are presented in Table I. In these calculations samples 15–23, 27 and 37 which were C.P. sal qualities were not included. Stunted trees or those of which quality classes were not known or were in doubt have been left out. The detailed data have been filed in Ecology Branch of the Forest Research Institute and can be readily had for examination.

TABLE I

Quality class of sal.	Foliar ash class indicated by number of samples						Mean % ash for each quality class	Total number of samples studied for each quality
	Ash Below 3%	Ash 3–5%	Ash 5–7%	Ash 7–9%	Ash 9–11%	Ash Above 11%		
I	2	13	1	..	..	..	3·92	16
II	1	15	6	..	..	..	4·20	22
III	..	21	3	3	2	1	5·34	30
IV	..	19	6	..	..	..	4·49	25

The statistical analysis of the data shows that differences in ash percentage of sal leaves for various quality classes are significant and quality III had significantly higher ash percentage than the other three qualities.

The present data show that in qualities I–III foliar ash increases but it shows a decrease in IV quality sal. Thus, it may seem possible to relate various quality classes of sal with foliar ash to some degree.

Although foliar ash in a tree is generally considered to be an index of its nutritional requirements it may also indicate the level of minerals in the soil and their availability under the existing conditions of climate. The differences in foliar ash in different qualities of sal in the present case may be related to the type of soil, though exact information on this point is at present lacking. Arrangements are being made to examine soil conditions under different quality classes studied so that relation between soil conditions and foliar ash be established. There is a possibility of getting a good correlation between soil Ca or pH and foliar ash, for Chandler (1941) has shown in American species that pH of the surface soil determines the amount of calcium in leaves of trees growing in such situations and similar data were provided by studies in English Woodlands (Puri, 1950).

It is interesting to note that in the same compartment on apparently similar type of rock and soil the three qualities of sal show differences in foliar ash (see numbers 33, 34 and 35; 18, 19 and 20; 21, 22 and 23; 24, 25 and 26) which are of the same type as the means. Taking samples from a single compartment it will be noted that foliar ash in I quality is not always lower than those of II and III.

These facts may indicate the probability of different qualities of sal in different parts of the country being eco- or even geno-types. This suspicion is further strengthened from the regeneration behaviour of the three qualities in natural forests. For example, in the Punjab and most parts of the Dun Valley, where sal is of III–IV quality, regeneration is usually scarce and difficult; though some I quality forests of the Uttar Pradesh are also giving difficulties in regeneration.

Our present knowledge of cyto-genetics and aut-ecology of sal is extremely limited; and even chromosome number of *Shorea robusta* is not known. This is true for many of the valuable timber species of India, *e.g.*, teak, *Terminalia tomentosa*, etc. (see Darlington and Janaki Ammal, 1945). For successful study of the regeneration problem of *Shorea robusta*, as also of any other species, it is desirable that information on these fundamental points be collected. In addition to these there is a great need for provenance studies of sal and other forest trees. A considerable amount of work has been done in Sweden by Langlet on spruce and pine. The results of these admirable studies were recently given in English by Lindquist (1948). The author has recently started an investigation on the germination behaviour and seedling vitality of seeds taken from top, bottom and middle parts of the crowns of I quality sal trees from various divisions of U.P.

The examination of data for seasonal variations in foliar ash for different quality classes in the same compartment (*e.g.*, Amguri 19)

shows that mature sal leaves of all quality classes collected in the month of November 1949, have higher amounts of ash while in still older leaves collected in the month of January 1950, the percentage of ash is somewhat low for each quality class as is seen in Table II below.

TABLE II

Registered Nos. of the samples	Date of collection	Quality	% Ash	Difference between same quality in % ash
69	17 Nov. 1949	I	3·90	0·55
163	12 Jan. 1950	I	3·35	
68	17 Nov. 1949	II	5·30	1·93
154, 155	12 Jan 1950	II	3·60, 3·15	
73, 70, 71	17 Nov. 1949	III	5·10, 4·35, 4·20	0·57
157, 186, 158	12 Jan. 1950	III	4·62, 3·75, 3·57	

Lutz and Chandler (1946) have shown that in white pine, percentage of ash and CaO increases with age. In *Quercus alba*, *Q. borealis* and *Q. montana* (*loc. cit.*, p. 151) it is reported that percentage of foliar Ca, N, P and K increases with age from May to September. Data for nine hardwood species compiled from various sources by the same authors (*loc. cit.*, p. 150) show that from June to October percentages of foliar ash, Ca and Mg increases with age, while percentages of P, K, S and N decreases.

These facts are of considerable interest from the point of natural economy of essential minerals and plant foods in a forest. From the known data of American authors quoted above, it would seem that plant litter normally returns to the soil much of Ca and Mg while much of P, K and N is not returned. These substances govern the growth of seedlings in the forest. The exact knowledge of these changes is therefore, of great importance in forest regeneration and succession studies. To fill this gap in our knowledge, weekly data for 10 Indian species including sal growing in the Demonstration Area at the Forest Research Institute are being collected since 3rd April 1950.

SUMMARY

The examination of foliar ash in I–IV quality classes of sal from different parts of the country have shown that ash content in these qualities is significantly different. It increases in qualities I–III and then shows a decrease in IV quality. These results agree with my earlier observations and those of others that sal is a non-exacting species, showing its best development on non-calcareous, acidic soils but too high acidity in the soil is unfavourable for its growth.

Literature Cited

Champion, H. G. 1933. Regeneration and management of sal (*Shorea robusta*). Ind. For. Rec., 19 (3): 1–200.

Chandler, R. F. 1941. The amount and nutrient content of freshly fallen leaf litter in the hardwood forests of central New York. Journ. Amer. Soc. of Agronomy. 33: 859–871.

Darlington, C. D. and Janaki Ammal, E. K. 1945. Chromosome atlas of cultivated plants, London.

Griffith, A. L. and Gupta, R. S. 1947. The determination of characteristics of soil suitable for sal (*Shorea robusta*). Ind. For. Bull. 138.

——— and Sant Ram. 1943. Yield and stand tables for sal (*Shorea robusta* Gaertn, f.) high forest. Indian For. Rec. Silvi. *New Series*, Vol. 4–A (4): 171–287.

Lindquist, B. 1948. Genetics in Swedish forestry practice. The Chronica Botanica Co., Waltham.

Lutz, H. L. and Chandler, R. F. 1946. Forest Soils, New York, p. 147.

Maitland, V. K. 1924. Volume tables for (*Tectona grandis*) teak and (*Shorea robusta*) sal for the Central Provinces. Ind. For. Rec. 11 (7): 215–222.

Puri, G. S. 1950. Ecology of the humus layer in some english forests. Ind. For., 76 (10, 11): 418–427 ; 453–466.

———. 1950. Soil pH and forest communities in the sal (*Shorea robusta*) forests of the Dehra Dun Valley, U.P., India. Ind. For., 76 (7): 292–309.

——— and Gupta, A. C. 1950. The calcium content of the foliage of sal and its common associates in the Dun Valley. Journ. Ind. Bot. Soc. 29: 139–144.

Troup, R. S. 1921. Silviculture of Indian Trees, Vol. I, London.

THE EMBRYOLOGY OF *MUNTINGIA CALABURA* L.

By C. Venkata Rao

Department of Botany, Andhra University, Waltair

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The family Elæocarpaceæ, to which *Muntingia calabura* L. belongs, consists of 7 genera and about 120 species (Willis, 1948). Bentham and Hooker, Hutchinson (1926), Edlin (1935) and Rendle (1938) treat this as a tribe of Tiliaceæ, while Engler and Prantl make this a separate family, mainly on anatomical grounds. The triangular vascular rays and phloem strands (stratified into fibrous and non-fibrous zones) which are characteristic of all other families of Malvales (namely, Scytopetalaceæ, Sterculiaceæ, Tiliaceæ, Bombacaceæ and Malvaceæ), as also the mucilage canals and sacs, are absent in the members of Elæocarpaceæ. Metcalfe and Chalk (1950) are of the opinion that "the anatomical structure of this family is sufficiently distinct from that of Tiliaceæ to justify treating this family separately". Kukachka and Rees (1943) who made a special study of the anatomy of the wood of the members of Elæocarpaceæ as well as Tiliaceæ, think that practically all the anatomical characters of Elæocarpaceæ point to its being the more primitive.

Previous Work

The only embryological work done on this family is that of Mauritzon (1934), who studied megasporogenesis and development of the embryo-sac in two species of *Aristotelia*, namely, *A. racemosa* and *A. maqui*. He also made a few observations on the early stages of development of the endosperm and seed coat. Nothing is known about microsporogenesis, pollen grains and embryo development in this family.

According to Mauritzon (1934), the development of the embryo-sac in *Aristotelia* conforms to the *normal*-type. Two archesporial cells are frequently met with in *A. racemosa*; these function up to the early stages of the embryo-sac. Linear as well as T-shaped tetrads are found in both species. The third megaspore from the micropylar side is reported to function frequently. The two polar nuclei fuse just before fertilisation. The antipodals are ephemeral and the cytoplasm of the embryo-sac shows starch grains. Mauritzon found an aggressive enlargement of the embryo-sac soon after fertilisation. This is more prominent in *A. maqui*; the embryo-sac crushes the whole of the nucellus and reaches the inner epidermis of the inner integument. The ovules are crassinucellate, anatropous and show a zig-zag micropyle, which is formed by both the integuments. There is a chalazal outgrowth on the ovules of both species of *Aristotelia*.

FIGS. 1–10. *Muntingia calabura*.—Fig. 1. T. S. of a young anther-lobe. Fig. 2. Tapetum and a tetrad of microspores. Fig. 3. Mature pollen grain. Fig. 4. External view of a germ pore. Fig. 5. A portion of the wall of a mature anther showing fibrous endothecium. Fig. 6. T. S. of a part of the ovary showing the placentæ, vascular bundles and groups of stone cells (dotted). Fig. 7. L. S. of ovary of an open flower showing the pendulous placentæ, the ovule primordia and the transmitting tissue in the stylar region and germinating pollen grains on the stigma. Fig. 8. L. S. of a young ovule showing megaspore mother cell. Fig. 9. L. S. of a fertilisable ovule. Fig. 10. The chalazal part of a mature ovule showing the socket of thick walled cells around the embryo sac and the hypostase-like strand. Fig. 1, × 425; Figs. 2 and 3, × 1070; Fig. 5, × 285; Figs. 6 and 7, × 30; Fig. 8, × 285; Fig. 9, × 180; Fig. 10, × 425.

Materials and Methods

The present paper deals with the life-history of *Muntingia calabura* L. which is grown in some South Indian gardens for its profuse blossoms. Its pulpy fruits are also edible. The material for the present investigation was obtained from the plants cultivated at Kakinada. It was fixed in formalin-acetic-alcohol with satisfactory results and studied according to the customary methods.

The Flower

The flowers of *Muntingia calabura* L. are axillary and pentamerous, with valvate calyx and imbricate corolla. To the inside of the numerous free stamens are found many glandular hairs, which are probably concerned in the secretion of honey. The ovary is superior, 5-carpellary, syncarpous and raised on a gynophore. It tapers at the top and terminates in a 5-radiate capitate stigma. There are two pendulous placentæ in each loculus on the surface of which are found numerous ovules. The placentæ look C-shaped in transverse section (Fig. 6). In the younger stages the placentæ appear straight in longitudinal sections (Fig. 7), but as they continue their growth even after the ovary has ceased to grow, their tips become curved and bend upwards.

Microsporangium and Male Gametophyte

The archesporium consists of 2–3 rows of hypodermal cells in each of the four lobes of the anther primordium. The anther wall eventually becomes 5 cells thick (Fig. 1). Of these, the cells of the epidermal layer enlarge considerably and accumulate early some deep staining contents. These progressively decrease as the fibrous thickenings develop in the sub-epidermal endothecial layer. The tapetum is derived from the innermost wall layer and is of the secretory type as in Tiliaceæ and the Dombeyeæ of Sterculiaceæ (Rao, 1949). The cells become binucleate and the cytoplasm gets vacuolated (Fig. 2). They persist till the pollen grains are fully formed.

By repeated mitotic divisions, the primary sporogenous cells form a large number of microspore mother cells; about 12–15 cells are seen in transverse sections of the anther loculus and 35–40 in a row in longitudinal sections. The two meiotic divisions proceed normally and result in tetrahedrally arranged tetrads of microspores. Cytokinesis occurs by furrowing. The young tetrad is invested by a callose sheath (Fig. 2).

Mature pollen grains are small and measure about 10 μ in diameter. They are spherical. The exine is reticulately thickened and shows a collar-like thickening around each of the three equatorially arranged germ pores, as was also seen in Dombeyeæ (Rao, 1950 *a*). The intine is uniformly thin and protrudes slightly through the germ pores as in *Waltheria indica* (Rao, 1950 *a*). The pollen grains are shed at the 2-nucleate stage (Fig. 3) and the division of the generative cell occurs inside the pollen tube as in most Malvales.

Ovule and Integuments

The ovules are anatropous and bitegmic. Except those at the base and top of the loculus, which are variously inclined, they are placed with their bodies transverse to the axis of the ovary (Fig. 6). The initials of both the integuments arise simultaneously, but the outer integument begins to grow faster as the archesporium differentiates in the ovule primordium. It closes up by the time the megaspore mother cell is ready to divide (Fig. 8), as is also the case in several members of Sterculiaceæ and Tiliaceæ. Ultimately both the integuments form the micropyle, which has the characteristic zig-zag form of the Malvales. In the mature ovule, the outer integument is 2 cells thick all over except in the region of the micropyle, while the inner is uniformly 3 cells thick (Fig. 9).

The nucellus is straight and massive; in the fertilisable ovule, there are 3–4 cell layers above and to the sides of the embryo-sac and 10–12 below it. As in *Aristotelia*, the nucellar epidermis does not form an epidermal cap, which is commonly seen in several genera of Sterculiaceæ and Tiliaceæ. Early in the development of the embryo-sac, 1 or 2 layers of cells around its extreme antipodal end become thick-walled and prevent its further growth. In this feature and consequently in the shape of the mature embryo-sac, *Muntingia* resembles genera like *Pterospermum* (Rao, 1949) and *Melochia* (Rao, 1951). In course of time, the cells between this socket and the vascular bundle in the chalaza also become thick-walled and form a hypostase-like strand about 3 cells in thickness (Fig. 10). In this feature *Muntingia* resembles *Waltheria* (Rao, 1950 *b*) and *Tilia* (Stenar, 1925). The ovules develop a chalazal outgrowth, which is also supplied by the vascular strand (Fig. 9).

Mucilage sacs and canals which are present in other genera of Malvales are absent from the ovary and ovules of *Muntingia* in the younger stages, but after fertilisation isolated cells of the septa become filled with dark-staining mucilaginous contents. The zone of tannin-bearing cells which forms a conspicuous feature of the fertilisable ovules of several Malvales becomes recognisable only after fertilisation. These cells become thick-walled and stand out prominently in the seed (Fig. 44).

Ovules with double nucelli, possessing a separate inner but a common outer integument, similar to those found in *Pterospermum acerifolium* (Rao, 1949) were also noticed occasionally.

The growth of the ovules in *Muntingia* deserves special mention. Usually in other Malvales, the blossoming of the flower synchronises with the attainment of the fertilisable stage by the ovules. In *M. calabura*, however, when the flower opens the ovules are still in the stage of very small primordia, although the stigma soon becomes receptive and the pollen grains lodged on it begin to germinate (Fig. 7). Fertilisation takes place after an interval of 12–15 days. To ascertain this, about 20 flowers which blossomed on one day were labelled and the developing ovaries were fixed one on each successive day. When these were microtomed, they showed the following stages.

(There is a slight difference in the stage of development of the ovules, but it is negligible. Usually the ovules towards the top of the loculus are at a more advanced stage.)

No. of days after flowering	Size of the ovary in mm.	Average size of ovules in microns	Stage of development of the ovules
Early on the day of flowering	3·8 × 2	70	Archesporium and integument initials demarcated.
Late on the same day	3·8 × 2	75	Primary parietal cell cut off.
2nd day ..	4 × 2	80	Outer integument more than half the height of nucellus; parietal cell divides.
3rd day ..	6 × 3·5	110	Parietal tissue two-layered.
4th day ..	7 × 4·2	130	Parietal tissue 3–4-layered.
5th day ..	7 × 4·5	150	5–6-layered parietal tissue; megaspore mother cell nearly full grown.
6th day ..	7 × 5·5	165	Full grown megaspore mother cell; 6–7-layered parietal tissue; stray dyads.
7th day ..	7·5 × 5·5	180	Megaspore mother cells, dyads and tetrads.
8th day ..	7·2 × 5·8	200	Enlarging functional megaspores and 2-nucleate embryo-sacs.
9th day ..	7·5 × 5·8	210	2-, 4-, or 8-nucleate embryo-sacs.
10th day ..	7·5 × 6	225	Young and fully organised embryo-sacs.
11th day ..	7·5 × 6	230	Mature embryo-sacs; stray pollen tubes.
12th day ..	7·8 × 6	240	Mature embryo-sacs; pollen tubes frequent.
13th day ..	8 × 6·1	265	Pollen tubes abundant; fertilisation stages.
14th day ..	8·1 × 6·3	280	Fertilisation stages; primary endosperm nucleus formed.
15th day ..	8 × 6·5	310	Division of the primary endosperm nucleus.
16th day ..	8 × 6·5	340	A few endosperm nuclei formed.
17th day ..	8·2 × 6·8	360	Fertilised egg.
20th day ..	8·4 × 7·1	375	Oospore not yet divided.

From the above table it is clear that the growth of the ovary and ovules is continuous and gradual up to the time of fertilisation and

that the ovules with the megaspore mother cell grow for a comparatively long time as was also found in many members of Sterculiaceæ (Rao, 1951). The occurrence of megaspore mother cells, dyads and tetrads in the ovule of the same loculus, shows that the two meiotic divisions proceed rather rapidly.

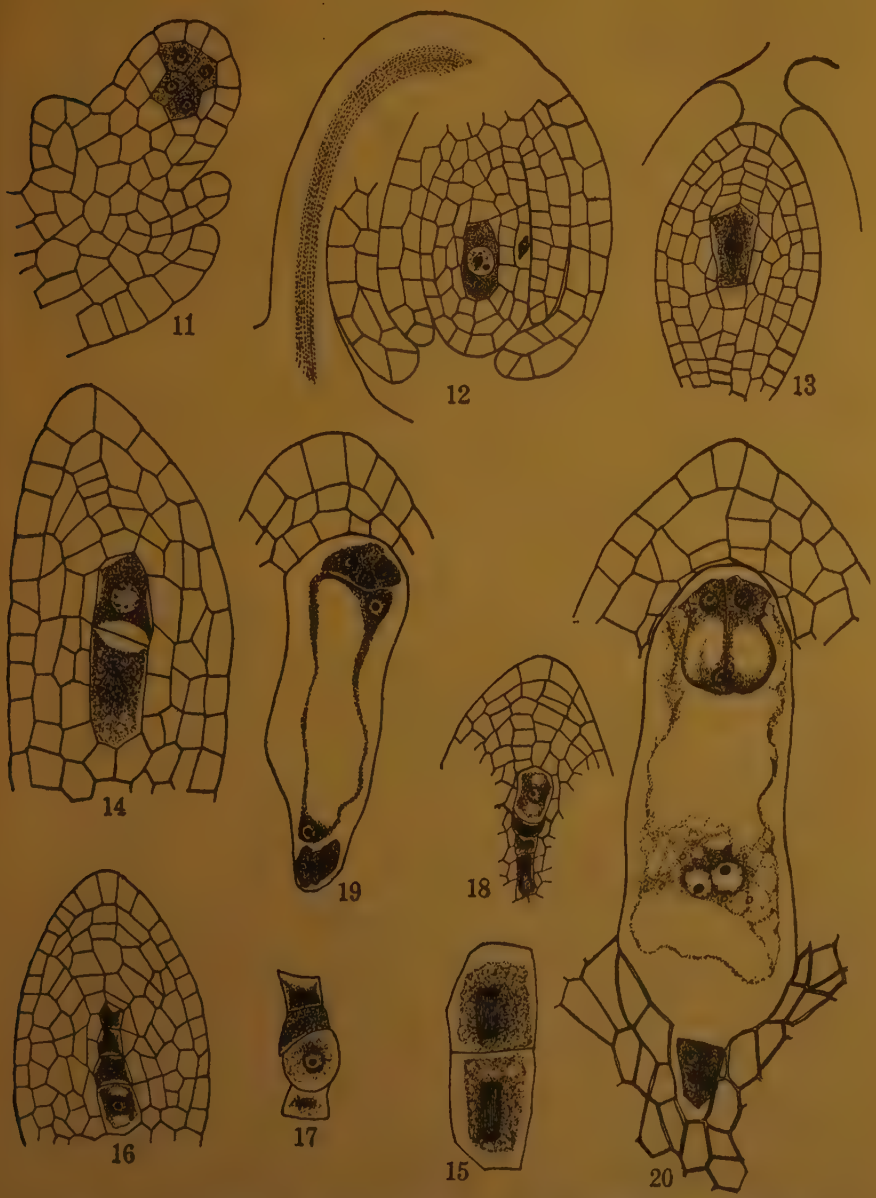
Though stray pollen tubes are noticed in ovaries fixed 11 days after blossoming, they are abundant only after 13 days. Such long intervals between pollination and fertilisation are noticed in Amentiferous genera like *Alnus*, *Corylus* and *Quercus* (Benson, 1894). Goebel associated this phenomenon with the woody habit (Coulter and Chamberlain, 1904, p. 147), but it is also seen in orchids. In *Paphiopedilum maudiæ*, for instance, the interval is about 5 months and the ovules develop only after pollination (Duncan and Curtis, 1942). One common feature of orchids and *Muntingia* is the presence of a very large number of ovules in the ovary. In the opinion of the writer, there seem to be two advantages in this phenomenon. Firstly, the separation in time of the development of the pollen grains and ovules of the flower results in an equitable distribution of food materials. Secondly, the early pollination gives enough time for the development of the large number of pollen tubes which are necessary for the fertilisation of the numerous ovules. Thus the number of unfertilised ovules and unnecessary wastage is minimised.

Megasporogenesis and Female Gametophyte

Though the archesporium is multicellular to start with (Fig. 11), as a rule only one axially placed hypodermal cell functions and the rest merge into the nucellus. Two functional archesporial cells were noticed only in early stages of development, *i.e.*, up to the megaspore mother cell stage. In such cases, they are collateral in position.

By a periclinal division of the archesporial cell, the primary parietal cell is formed to the outside and the megaspore mother cell to the inside. The first division of the primary parietal cell is anticlinal; ultimately the derivatives of this cell form 5-6 layers of more or less regularly arranged cells below the nucellar epidermis (Figs. 13 and 14). In later stages some of these get crushed by the enlarging embryo-sac. The full grown megaspore mother cell has an elongated and slightly tapering form (Fig. 13). Usually the nucleus lies about the middle of the cell, but sometimes it was seen to stand a little nearer to the micropylar end of the cell. Due to the variations in position of the nucleus at the time of division there is a corresponding difference in the size and shape of the dyads and megaspores. Either all of them are of nearly the same size or the lowest cell is a little longer than the others. The two dyad cells of an ovule may divide simultaneously (Fig. 15) or the division in the lower dyad may precede that in the upper (Fig. 14). In any case, as the spindles are always oriented parallel to the long axes of the cells the megaspores are arranged in a linear fashion. About 50 tetrads were examined, but T-shaped tetrads were not encountered in *Muntingia*.

Usually the lowest megaspore of the tetrad is functional. In one exceptional instance the first megaspore (Fig. 18), and in another the



FIGS. 11–20. *Muntingia calabura*.—Fig. 11. Ovule primordium showing multi-cellular archesporium. Figs. 12–15. Various stages in the formation of megaspore tetrad and parietal tissue. Fig. 16. Ovule with the chalazal megaspore of the tetrad functioning. Fig. 17. A megaspore tetrad in which the third megaspore is enlarging. Fig. 18. A megaspore tetrad in which the first megaspore is functioning. Figs. 19 and 20. Young and mature 8-nucleate embryo sacs. Figs. 11, 14, 19 and 20, × 690; Figs. 12, 13, 16 and 18, × 425; Figs. 15 and 17, × 1070.

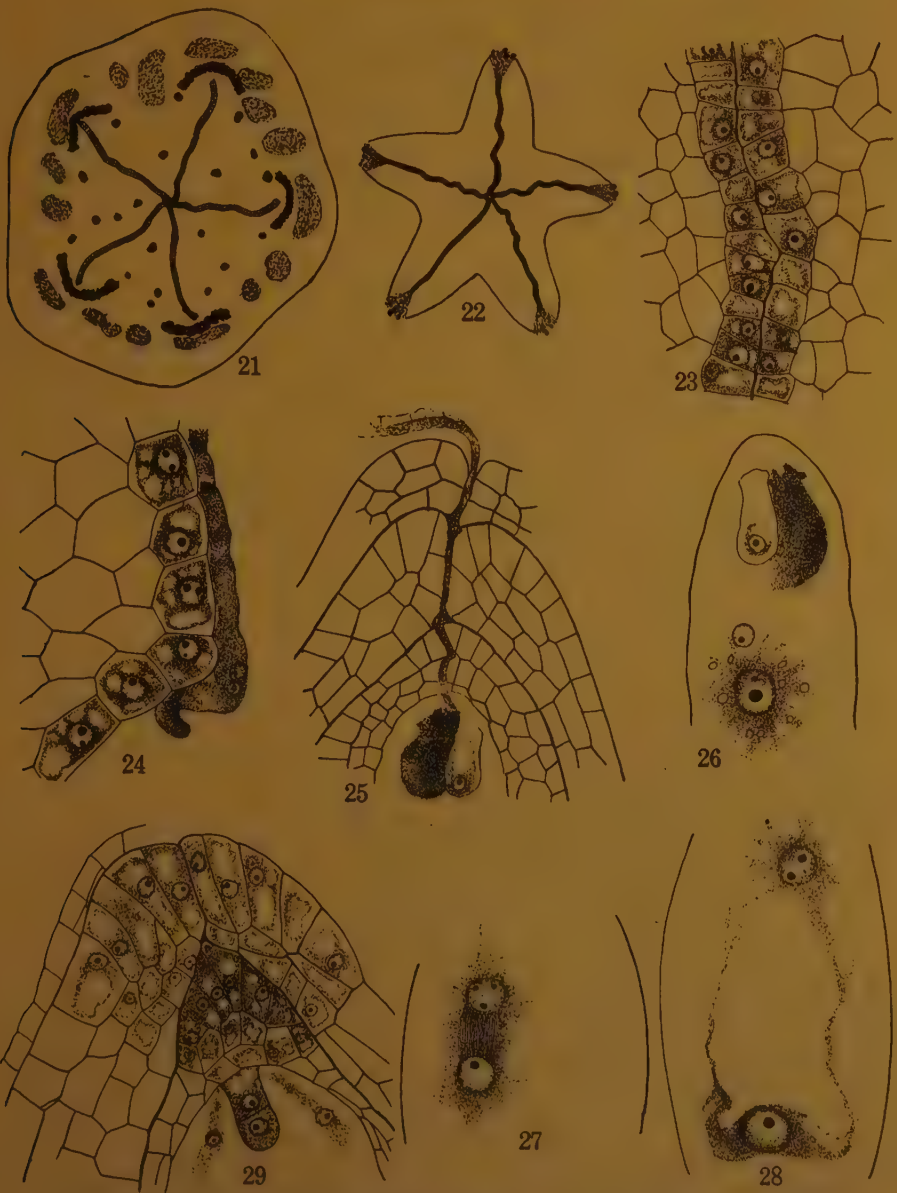
third megaspore from the micropylar end (Fig. 17) were seen to be enlarged while the rest were degenerating. Hence the T-shaped tetrads or the functioning of the third megaspore, which are commonly noticed in *Aristotelia* (Mauritzon, 1934), cannot be taken to have any systematic significance.

By three successive free nuclear divisions in the functional megaspore, the 8-nucleate embryo-sac is formed. Cell walls are organised around the antipodals earlier than around the egg apparatus (Fig. 19). The embryo-sac presents normal features (Fig. 20). The upper polar nucleus descends and meets its partner which till then remains stationary. The two then travel upwards and fuse in the middle of the sac, just before fertilisation as in *Aristotelia*. By this time, the antipodals which are richly protoplasmic at the beginning, degenerate completely. In the formation of a secondary endosperm nucleus, *Muntingia* resembles genera of Bombacaceæ like *Eriodendron* (Thirumalachar and Khan, 1941) and *Bombax* (Banerji, 1942) and some Malvaceæ (Stenar, 1925) and differs from Sterculiaceæ and Tiliaceæ. The synergids show hooks on their free sides and prominent vacuoles at their chalazal ends and differ from the synergids of *Aristotelia* in which hooks are reported to be absent and the cytoplasm fills the cells uniformly. A few starch grains are seen in the cytoplasm of the mature embryo-sac (Figs. 20 and 26).

Fertilisation

As already described, there are two placentæ in each loculus hanging from the top of the septa. The epidermal cells of the funicles of fertilisable ovules as well as those of the placentæ are glandular and richly protoplasmic (Fig. 23). A little above the attachment of the placentæ, the septa split radially and a 5-angled axial canal appears, but this soon closes up. The epidermal layers of the placentæ extend upwards to the stigma running in close contact with each other. In the terminal region of the ovary, which is functionally the style, though it is not marked off by any constriction, these appear in tranverse sections as 5 V-shaped zones (Figs. 21 and 22). They constitute the transmitting tissue. The cells of this tissue are thin-walled and richly protoplasmic, isodiametric in the stylar region (Fig. 23), but somewhat elongated just below the stigma. The vascular bundles (and groups of stone cells) of the ovary run close to this tissue and keep it well supplied with food (Fig. 21). This continuous system of richly protoplasmic cells, which extend from the stigma to the micropyles of the ovules, seems to be well adapted to nourish the large number of pollen tubes which traverse it during the long interval between pollination and fertilisation.

As the flower opens, the pollen grains are lodged in large numbers in the stigmatic furrows, which are kept moist with a mucilaginous secretion. Though initially the intine protrudes through all the three germ pores, the emergence of the pollen tube is monosiphonous. The tube takes an intercellular course between the two layers of the transmitting tissue and after reaching the loculus, passes along the surface of the glandular cells of the placentæ and funicle (Fig. 24). It enters the ovule in a porogamous manner and empties its contents into a



FIGS. 21–29. *Muntingia calabura*.—Fig. 21. T. S of style showing transmitting tissue, vascular bundles (deeply shaded) and stone cells (dotted). Fig. 22. T. S. of stigma with germinating pollen grains in the furrows. Fig. 23. Portion of the transmitting tissue magnified. Fig. 24. Glandular epidermal cells of the placenta with the pollen tube on the surface. Fig. 25. Entry of the pollen tube into the ovule. Fig. 26. A stage in fertilisation. Figs. 27 and 28. Division of the primary endosperm nucleus. Fig. 29. The micropylar part of the ovule after fertilisation. Figs. 21 and 22, × 30; Figs. 23, 24, 26, 27 and 28 × 690; Figs. 25 and 29, × 425.

synergid, as was also noticed in *Waltheria* (Rao, 1950 *b*) and *Melochia* (Rao, 1951). Consequently the synergid enlarges considerably and gets filled with deep staining contents (Fig. 25). The unaffected synergid degenerates soon after. Occasionally two pollen tubes were seen in a nucellus and sometimes both penetrated into an embryo-sac, destroying the two synergids as was also noticed in *Melochia* (Rao, 1951). The synergid that receives the pollen tube later on bursts at the chalazal end and liberates the two male nuclei, the tube nucleus having degenerated earlier.

Endosperm

As in *Aristotelia*, in *Muntingia* also there is a rapid enlargement of the embryo-sac soon after fertilisation. An embryo-sac which is being penetrated by the pollen tube measures about 90 μ by 25 μ, while the same with fertilised egg measures 160 μ by 50 μ. This increase in the size of the embryo-sac takes place at the expense of the nucellus, but due to the presence of the socket of the thick-walled cells, the whole of the nucellus is not crushed at this stage as in *Aristotelia*. A large part of it persists until the endosperm becomes cellular and the embryo reaches the quadrant stage.

The primary endosperm nucleus seems to divide within 24 hours of its formation, because it is often seen that in the same ovary while some ovules may show fertilisation stages, others may show 2 or 4 endosperm nuclei. The primary endosperm nucleus divides while still situated about the middle of the embryo-sac, the spindle being formed parallel to the long axis of the sac (Fig. 27). Of the two nuclei formed, one migrates to the antipodal end. Close observation of the early stages of the endosperm development showed that the cytoplasm around the endosperm nucleus at the antipodal end of the sac is richer and more deep staining (Fig. 28). Nuclear divisions also proceed more vigorously in this region so that there arises early an antipodal accumulation of endosperm nuclei (Fig. 30), which seems to facilitate the rapid transport of food materials into the sac from the chalazal end.

In addition to the above, there is another device that seems to help in nourishment of the young embryo. Soon after fertilisation, the placental tissue swells considerably due to the enlargement of the parenchymatous cells that compose it. The cells lose their integrity and their walls become extremely thin and take very little stain. This disintegrating mass of cells seems to provide food material, which the large number of rapidly developing seeds need. The vascular tissue in the axial region of the ovary also undergoes marked secondary thickening due to the activity of a cambial ring. This also seems to be associated with the nutritional demands of the growing seeds and fruit.

During this period, the cells of the outer epidermis of the inner integument in the micropylar region become radially elongated. In the fertilisable ovules, the length of these cells is 10–15 μ, while in the ovules with fertilised eggs, they measure 30–35 μ. They show thin

walls and vacuolated cytoplasm. The cells of the apical region of the nucellus also become conspicuous at this time by their radial elongation, richer cytoplasm and deeper stain than the neighbouring cells (Fig. 29). They form a wedge-shaped protuberance which crushes some of the cells of the inner integument and abuts on its radially elongated epidermal cells. This series of richly protoplasmic cells extending to the embryo seems to help in its nutrition with food available from placental tissue (Fig. 29).

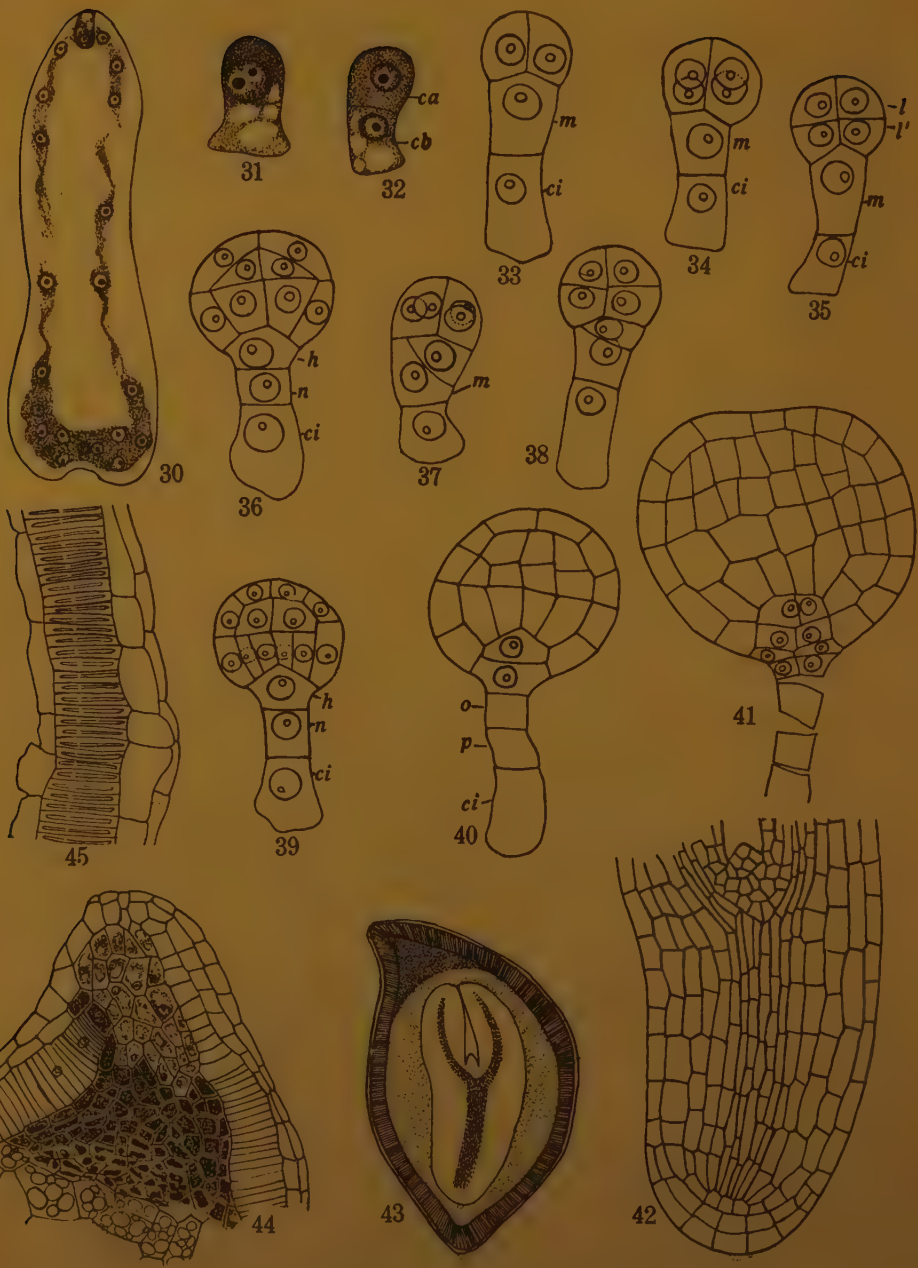
Embryo

In *Muntingia* the development of the embryo keys out to the *Onagrad* type of Johansen (1950).

After a number of endosperm nuclei have been formed, the fertilised egg divides transversely and gives rise to the basal (*cb*) and terminal (*ca*) cells (Fig. 32). While the basal cell is provided with vacuolated cytoplasm the terminal cell shows dense cytoplasm and is somewhat globular. The second division occurs simultaneously in both the cells: *ca* divides longitudinally and *cb* transversely, producing the lower cell *ci* and an upper cell *m*, which functions as the hypophyseal initial (Fig. 33). Next, the two terminal cells undergo another longitudinal division in a plane perpendicular to that of the previous division, giving rise to circum-axially arranged quadrants (Fig. 34). Then the quadrants undergo a transverse division to give rise to octants arranged in two tiers of four cells each, *l* and *l'* (Fig. 35). (Later, *l* gives rise to the cotyledons and stem tip and *l'* to the hypocotyl region). At this stage, the cell *ci* has enlarged and become somewhat vesicular and from its appearance in subsequent stages it does not seem to undergo any division. Cell *m* with its upper end projecting into the embryonal mass has the usual form of "gabled roof". After the octants undergo periclinal division and the dermatogen initials are demarcated to the outside, *m* divides in a transverse manner and forms the upper cell *h* which functions as the hypophysis and a lower cell *n* which adds to the suspensor (Fig. 36). Later, the cell *n* undergoes a transverse division and forms cells *o* and *p*, due to which the suspensor becomes 3-celled (Fig. 40), as was also noticed in *Waltheria* and *Triumfetta*.

The hypophyseal cell undergoes a transverse division in which a curved cell wall is formed between the two daughter cells. Therefore, as usual, the upper cell has a lenticular shape and the lower one is like a watch glass (Fig. 40). The upper cell gives rise to the root tip of the embryo and the lower functions as the root cap initial.

In a few cases, cell *m* was seen to divide in a manner different from the usual type described above (Figs. 37 and 38). Instead of the wall being formed transversely, it was laid in an inclined manner so that both the daughter cells had part of their bodies in contact with the embryonal mass. The division in such cases in *m* occurred quite early, even at the quadrant stage of the embryo, while normally it divides only after the dermatogen initials are formed in the octants. Such abnormalities are also reported in *Eulobus sceptrostigma* of Onagraceae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FIGS. 30–45. *Muntingia calabura.*—Fig. 30. Embryo sac with fertilised egg showing antipodal accumulation of endosperm nuclei. Figs. 31–41. Various stages in the development of the embryo. Fig. 42. L. S. part of the mature embryo showing the root cap, root apex, hypocotyl and plumule. Fig. 43. L. S. of a mature seed. Flg. 44. Zone of tannin-bearing cells in the chalaza. Fig. 45. L. S. of the seed coat showing the membraneous sheath of two layers formed by the outer integument, the palisade layer formed by the outer epidermis of the inner integument and the remnants of the remaining layers of the inner integument. Figs. 31–41, × 690; Fig. 42, × 200; Fig. 43, × 75; Fig. 44, × 235; Fig. 45, × 425.

(Johansen, 1950, p. 168), in which the hypophyseal cell is derived by the formation of another inclined wall in the upper daughter cell.

The mature embryo is straight with well-developed cotyledons and stem tip (Figs. 42 and 43). It is surrounded by about three layers of endosperm cells, but there is no perisperm. Like the cells of the endosperm, the cells of the embryo, including those of the plerome strands, are packed with starch grains.

SEED COATS

The development and structure of the seed coats are very similar to those of other families of Malvales. The outer integument remains two cells thick and forms only a membraneous sheath. The inner becomes 4–5 cells thick after fertilisation. Its outer epidermis develops into the palisade layer with radially elongated cells, in which the 'light line' is distinctly seen (Fig. 45). During the early stages, the cells of the sub-epidermal layer become very large and isodiametric and accumulate some deep staining contents which get diminished as the thickenings in the walls of the palisade cells become more and more prominent. Ultimately all the layers of the inner integument except the palisade layer get crushed completely. Mauritzon (1934) noticed in *Aristotelia* species that the outer integument remains of the same thickness throughout its life, the inner (originally 6–7 layered) becomes 13–15 cells thick in *A. maqui* and 20–21-layered in *A. racemosa.* In *Muntingia,* on the other hand, the post-fertilisation increase in the number of cell layers in the inner integument is not so prominent.

SUMMARY

The anther of *Muntingia calabura* L. is 2-celled and has a 5-layered wall. The tapetum develops from the innermost wall-layer and is of the secretory type. Mature pollen grains are spherical and smooth-walled and possess three germ pores. They are 2-nucleate at the time of shedding.

The ovules are crassinucellate, bitegmic and anatropous. Both the integuments form the micropyle, which is zig-zag. A socket of thick-walled cells around the antipodal end of the embryo-sac and a hypostase-like strand below it are organised in the nucellus. A zone of tannin-bearing cells becomes conspicuous in the chalaza after fertilisation.

The ovules have usually only one functional hypodermal archesporial cell. A primary wall-cell is cut off, which forms 6–7 layers of

parietal tissue. No epidermal cap is formed. Megaspore tetrads are linear and the lowest megaspore functions and forms an embryo-sac according to the *normal* type. The antipodals are ephemeral and degenerate by the time the polar nuclei fuse.

There is an interval of 12–15 days between pollination and fertilisation. When the flower blooms the ovules are still very young. Fertilisation is porogamous; the pollen-tube empties its contents into a synergid which later bursts to liberate the two male gametes. Occasionally two pollen-tubes enter an embryo-sac.

Endosperm is nuclear to start with, but becomes cellular at about the quadrant stage of the embryo. There is formed an early accumulation of endosperm nuclei at the antipodal end of the sac. This seems to help in the nutrition of the embryo. The epidermal cells of the inner integument and the terminal part of the nucellus become radially elongated and serve in the rapid transportation of food materials which the disintegrating placental tissue provides.

The development of the embryo conforms to the *Onagrad* type. The mature seed is endospermic but without perisperm. The outer integument forms a membraneous testa and the palisade layer is derived from the outer epidermis of the inner integument as in other Malvales.

Acknowledgements

I wish to express my grateful thanks to Prof. A. C. Joshi for kind encouragement throughout the progress of the work. My thanks are due to Prof. J. Venkateswarlu for some valuable suggestions and also to Mr. L. L. Narayana, B.Sc., for kindly fixing the material for me.

Literature

Banerji, I. 1942. Development of the female gametophyte and floss in *Bombax malabaricum*. Proc. Ind. Acad. Sci. B. 16: 205–211.

Benson, M. 1894. Contribution to the Embryology of Amentiferæ. I. Trans. Linn. Soc. Bot. London, 3: 409–424.

Coulter, J. M. and Chamberlain, C. J. 1904. Morphology of Angiosperms. N. Y.

Duncan, R. E. and Curtis, J. T. 1942. Intermittant growth of fruits of *Cyprepedium* and *Paphiopedilum*. A correlation of the growth of orchid fruits with their internal development. Bull. Torrey. Bot. Club. 69: 353–359.

Edlin, H. L. 1935. A critical revision of certain taxonomic groups of the Malvales. New Phytol. 34: 1–20.

Hutchinson, J. 1926. Families of Flowering Plants. Vol. I. London.

Johansen, D. A. 1950. Plant Embryology. Waltham.

Kukachka, B. F. and Rees, L. W. 1943. Systematic anatomy of the woods of the Tiliaceæ. *Tech. Bull. Minn. agric. Exp. Sta.*, **158**: 1–70.

Mauritzon, J. 1934. Zur Embryologie der Elæocarpaceæ. Ark. Bot. 26: 1–8.

Metcalfe, C. R. and Chalk, L. 1950. Anatomy of Dicotyledons. Vol. I. London.

RAO, C. V. 1949. Contributions to the Embryology of Sterculiaceæ. I. Jour. Ind. Bot. Soc. 28: 180–197.

————. 1950 *a*. Pollen grains of Sterculiaceæ. Jour. Ind. Bot. Soc. 29: 130–137.

————. 1950 *b*. Contributions to the Embryology of Sterculiaceae. II. *Waltheria indica*. Jour. Ind. Bot. Soc. 29: 163–176.

————. 1951. Contributions to the Embryology of Sterculiaceæ. III. *Melochia corchorifolia* L. Jour. Ind. Bot. Soc., 30: 122–131.

STENAR, H. 1925. Embryologische Studien. I. Zur Embryologie der Columniferen. II. Die Embryologie der Amaryllidaceen. Acad. Abh. Uppsala.

THIRUMALACHAR, M. J. AND KHAN, B. A. 1941. Megasporogenesis and endosperm formation in *Eriodendron anfractuosum*. Proc. Ind. Acad. Sci. B. 14: 461–465.

WILLIS, J. C. 1948. A Dictionary of Flowering Plants and Ferns. VI Ed. Cambridge.

REVIEW

Fungi and Plant Disease. By B. B. Mundkur. London: MacMillan & Co., Ltd., 1949, 246 pp., 16 *s.*

This is a book primarily for students of agriculture and horticulture, but contains much useful information for post-graduate students of botany. It is divided into twelve chapters. Chapters I to V discuss in a general manner the morphology and reproduction of fungi, metabolic processes in fungi, diseases caused by fungi, methods of studying plant diseases, and fungal nomenclature. Chapters VI to X deal with the common diseases of major Indian crop plants caused by members of Phycomycetes, Ascomycetes, Basidiomycetes, Fungi Imperfecti and Bacteria. These include 12 separate diseases caused by Phycomycetes, 8 diseases caused by Ascomycetes, 14 diseases caused by Basidiomycetes, 9 diseases caused by Fungi Imperfecti and 3 diseases caused by Bacteria. Virus diseases are treated in Chapter XI, but the account is rather brief. A more elaborate treatment of the transmission of such diseases, the rôle played by insects in their spread and a detailed treatment of a few major virus diseases of Indian crop plants would have greatly added to the value of the book. The final chapter XII discusses methods of plant disease control.

The book is nicely printed and moderately priced, but it is not altogether free from errors. To state that ring disease (of Irish potatoes) "is soil borne" and that "tubers harvested from fields where disease is present invariably carry infection" is not correct. Rather the characteristic feature of this disease is its *lack of field spread or survival in the soil.* The terminology, in many cases, has been ambiguously used; to cite a few cases: a "zygote" is never "also called a synkaryon"; the male gamete of fungi is only rarely "known as an antherozoid"; antheridia of some Pythiaceous fungi are amphigynous and not the oospores as implied by the author.

Misprints occur at several places in the legends accompanying the text-figures: the word "leaves" has been used where only a single leaf is illustrated; the word "pustule" is written where a number of pustules are shown; "cleistothecia" is written where in reality a single cleistothecium is pictured; the inflorescence of *Colocasia* is erroneously indicated in the legend as a "flower".

Another very unusual feature is the citation of the scientific name of the host plant against the common name of the disease instead of the universal practice of indicating the binomial of the organism producing the disease.

J. C. Saha.

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